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Full Length Research Paper

Lectin isolated from Brazilian seeds of velvet bean (*Mucuna pruriens* (L) DC.) presents analgesic, anti-inflammatory and antihemolytic action

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Lectins are proteins present in all living beings capable of interacting specifically and reversibly to carbohydrates or glycoconjugates form. They stand out in this family of lectins legumes, which are of special scientific interest because they have different biological functions. This study investigated the presence of a lectin in Brazilian seeds of *Mucuna*, proceeding with their isolation and evaluation of its antinociceptive effects, anti-inflammatory and antihemolytic activity. Saline extracts of *Mucuna pruriens* were investigated for the presence of hemagglutinating activity by serial dilution and applied to Sephacryl S-200HR columns and DEAE Sephacel HiPrepFF to purify their lectin. After assessing the purity and molecular weight by polyacrylamide agarose gel electrophoresis (PAGE), the hemolytic activity on human erythrocytes was determined, as well as its antinociceptive action by models of writhing induced by acetic acid and formalin, in addition to its anti-inflammatory activity by testing the paw edema induced by carrageenan. The results indicated that Brazilian *Mucuna* seeds had a lectin that was purified from seeds having molecular weight of 60.0 kDa and only able to agglutinate erythrocytes of rabbit. The lectin showed an antihemolytic effect on human erythrocytes by not causing hemolysis in these cells compared to the negative control. The administration of lectin in rodents intraperitoneally inhibited the writhing by 99% (2.5 mg/kg), induced by acetic acid, as well as in the method where the formalin nociceptive stimulus was reduced by 51% in stage one and 77% in stage two of the test, using 2.5 mg/kg lectin. The anti-inflammatory activity demonstrated a decrease in paw edema induced by carrageenan in 72% better result than indomethacin control where there was a reduction of only 48% of edema. The study supports the presence of a lectin in Brazilian seeds of *M. pruriens* with antinociceptive, anti-inflammatory and antihemolytic activities.

Key words: Plant lectin, *Mucuna pruriens*, purification, antihemolytic, antinociceptive, anti-inflammatory.

INTRODUCTION

Lectins are a heterogeneous group of proteins that share an important biological propriety; they are capable of recognizing specific glycidic structures, interacting in a reversible way (Van Damme et al., 2008). They are found

in plants (Silva et al., 2012), vertebrates (Yang et al., 2014), invertebrates (Matsumoto et al., 2012) and microorganisms (Wu et al., 2010), frequently observed on the cell surface or intercellular particles (Lepenies et al., 2013). Among the kingdom Plantae they are abundant in seeds, roots, fruits, leaves and flowers, and are mainly obtained from ripe seeds of legumes in which they comprise up to 15% of total protein and may present one or more molecular forms (Loris et al., 1998).

The specificity of plant lectins concerning different carbohydrates makes possible their application on pharmaceutical research such as: target-cell recognition (Athamna et al., 2006), cellular adhesion, cell interaction, cell-matrix interactions (West and Goldring, 1994), fertilization and agglutination of cells and bacteria (Santi-Gadelha et al., 2012). Although possessing strong similarity on their physicochemical properties and three-dimensional structure, these biomolecules differ on their specificity to the carbohydrate they ligate and their physiological activity (Rego et al., 2002). Lectins already displayed activity on anti-neoplasia (Silva et al., 2014), apoptotic (Zhou et al., 2014), mitogenic (Wong et al., 2010), antibacterial, antifungal and insecticide (Vandenborre et al., 2011), anti-inflammatory (Santi-Gadelha et al., 2006), immunostimulant (Leite et al., 2012), among others. They are considered strong candidates for therapeutic use, for they are macromolecules with noticeable resistance to unfavourable conditions like pH and temperature variations and isotonicity, with no significant alterations to their biological function (Coffey et al., 1993).

Mucuna pruriens (L) DC. commonly known as velvet bean is a tropical legume member of the family Fabaceae, widely spread through the south and southeast regions of Asia (Duke, 1981). The seeds have been subject of study especially on their nutritional/ antinutritional contents (Machuka, 2000; Siddhuraju et al., 2000; Siddhuraju et al., 1996), as in India they are a common part of the diet of ethnical groups (Pugalenthi et al., 2005). Yet, there is no literature concerning the biochemical and pharmacologic properties that derivate from the lectins present in their seeds. Historically, its use on popular medicine is mainly associated to the treatment of depression, mental disorders and male infertility (Tripathi and Upadhyay, 2002). The *M. pruriens* seeds present high levels of L-DOPA, a drug applied on the treatment of Parkinson's, awakening growing interest on research within this species (Dhanasekaran et al., 2008; Lieu et al., 2010; Manyam et al., 2004; Yadav et al., 2013). However, most of the literatures available on the species' derive from pharmacological studies using aqueous extracts, making specific studies on the isolated bioactive compounds extremely relevant. In the present work, we report the isolation of a lectin present in Brazilian *M. pruriens* seeds, as well as the evaluation of its antinociceptive, anti-inflammatory and antihemolytic activities.

METHODOLOGY

Plants

The seeds of *M. pruriens* were collected in the city of João Pessoa, Paraíba, Brazil.

Animal subjects

We used male adult Swiss mice, with average body weight of 25 g, provided by the vivarium of Estácio School of Medicine of Juazeiro do Norte (Estácio/FMJ). The animals were kept on cages with free access to water and food, in a disturbance-free room with constant temperature (24°C) and 12-h light/dark cycle. FMJ's Institutional Committee of Animal Ethics approved the experimental protocol.

Erythrocytes

We obtained erythrocytes from rabbits of the Prof. George Thomas' vivarium, annex to the Biotechnology Center of Federal University of Paraíba (UFPB). The experimental protocol to obtain the cell samples was approved by the Institutional Committee of Animal Ethics of UFPB. We obtained human ABO system erythrocytes from expired transfusion bags donated by the state's blood bank, located in João Pessoa.

Lectin purification (MPLEC)

The whole seeds of *M. pruriens* were triturated and homogenized (1:10 m/v) via constant agitation during 3 h at 25°C, in NaCl 0.15 M. The extract obtained was then centrifuged for 20 min at 5000 rotation per minute (RPM) and 4°C. The supernatant went through water dialysis (remove NaCl 0,15 M) where the albumins (soluble in water and soluble in saline solutions) and the globulins (insoluble in water but soluble in saline solutions) fractions were separated using centrifugation under the same conditions before. The lyophilized albumin fraction was solubilized (100 mg/ml) in NaCl 0.15 M and applied in a molecular exclusion column Sephacryl S-200 HR HiPrep 26/60 with volume of 320 ml (GE Healthcare) and constant flow rate of 1.3 ml/min linked to AKTAprime plus system (GE Healthcare), the eluted product was monitored at 280 nm. The active peak (with hemagglutinant activity) obtained from the molecular exclusion, water-dialyzed, lyophilized and resuspended (40 mg/ml) in Tris-HCl 0.025 M pH 7.6 was applied to a 1 ml ionic-exchange column DEAE Sephacel HiPrep FF 16/10 (GE Healthcare) linked to a AKTAprime plus system (GE Healthcare). The protein fraction was eluted on Tris-HCl 0.025 M pH 7.6 with NaCl 0 to 1 M saline buffer gradient with constant flow rate of 1 ml/min and monitored at 280 nm. The protein was labelled MPLEC (*Mucuna pruriens* seed lectin). The purification's electrophoretic protein profile was determined by electrophoresis in polyacrylamide gel according to Laemmli (1970), at non-reductive conditions

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(PAGE), with the absence of beta-mercaptoethanol and sodium dodecyle sulphate (SDS), with 3% concentration gel and 12.5% separation.

Hemagglutinating activity and inhibition essays

We determined the MPLEC's hemagglutinating activity (UH) using human ABO and rabbit erythrocytes divided into two groups, processed and not processed by enzymatic treatment of trypsin, bromelain and papain according to Correia and Coelho (1995). UH was defined as the lowest dilution capable of causing hemagglutination. The hemagglutination inhibition essays to determine the lectin specificity were performed in the presence of glycoprotein (fucoidan, fetuin, mucin and λ-carrageenan) at initial concentration of 5 mg/ml and simple sugar carbohydrates (N-acetyl-D-glucosamine, D-glucose, saccharose, L-sorbose, D-fructose, α-lactose, arabinose, xylose, maltose, galactose, D-fucose, methyl-α-D-glucopyranoside, D-trehalose, mannose) at initial concentration of 0.5 M

Protein dosage

The soluble protein dosage was determined according to Bradford (1976), using bovine serum albumin as standard.

Molecular mass determination using PAGE

We determined apparent molecular mass using relative mobility (Mr = distance traversed by the protein compared to the distance traversed by the known molecular mass protein indicators: B phosphorylase 97.0 kDa; BSA, 66.0 kDa; ovalbumin 45.0 kDa; carbonic anhydrase 30.0 kDa; trypsin inhibitor 20.1 kDa; alpha-lactalbumin 14.4 kDa) compared to the relative mobility of MPLEC on PAGE (with the absence of beta-mercaptoethanol and SDS).

Hemolytic activity on human erythrocytes

We followed the protocol by Rangel (1997). Using human erythrocytes of the ABO system diluted in NaCl 0.15 M to achieve a 0.5% suspension, exposed to the lectin at 10, 100 and 1000 mg/ml concentrations. As a positive control, we applied the chemical hemolysant Triton X, and as negative NaCl 0.15 M. The solutions were submitted to agitation at 100 RPM for an hour at 25°C and centrifuged at 2000 RPM for 5 min. We read the liberated hemoglobin on a spectrophotometer at 540 nm. The lectin-induced hemolysis was calculated comparing to the Triton X treatment (100% hemolysis), and compared to the negative control.

Antinociceptive activity

Acetic acid-induced abdominal contortion model

Followed as determined by Vander Wende and Margolin (1956) for rats and modified by Koster et al. (1959) for mice. 30 min after administrating the lectin (MPLEC) via intraperitoneal injection (1 and 2.5 mg/kg), each mouse received an intraperitoneal injection of acetic acid 0.8% diluted in saline (0.1 mg/10 g). During the following 30 min, we counted the abdominal contortions presented by each animal. As positive control a group received Indometacine (Indom – 10 mg/kg, intraperitoneal) a standard analgesic drug, and as negative control we used saline (0.1 ml/10 g). All groups were constituted by seven animals treated independently.

Formalin model

This is done according to Hunskaar and Hole (1987) method. 30 min after administrating the lectin (MPLEC) via intraperitoneal injection (1 and 2.5 mg/kg), each mouse received 40 µl of formalin 1% (v/v, intraplantar) on the hind right foot. After, we registered the time the animal spent licking the injected foot during the initial 5 min (1st phase, neurogenic) and 20 min following (2nd phase, inflammatory). As positive control, a group was treated with Morphine (Mor – 5 mg/kg, intraperitoneal) a standard analgesic drug, and as negative control a group was treated with saline (0.1 ml/10 g). To verify the participation of opioid receptors on the pharmacological effect we used naxolone (Nalox – 2 mg/kg, subcutaneous), an opioid receptor's antagonist, 15 min before treatment with the lectin of morphine. All groups were constituted by seven animals treated independently.

Anti-inflammatory activity

Carrageenan-induced foot edema model

This was done according to Landucci (1995) method. 30 min after administrating the lectin (MPLEC) via intraperitoneal injection (1 and 2.5 mg/kg), each mouse received an intraplantar injection of 40 µl/foot of a solution of carrageenan 1% p/v on the left hind foot. Foot volume was measured by a plethysmograph (Ugo Basile, Italy), 1, 2, 3, 4, and 24 h after the treatment. Edema volume was calculated by the difference of initial and final foot volume. As positive control we used Indometacin (Indom – 10 mg/kg, intraperitoneal), a standard anti-inflammatory drug, and as a negative control we used a group treated with saline (0.1 ml/10 g, intraperitoneal). All groups were constituted by seven animals treated independently.

Statistical analysis

Results were presented by mean ± standard error of the mean (SEM). To detect statistical difference we applied the analysis of variance (ANOVA), followed by a Turkey's test, where $p < 0.05$ (* or #), $p < 0.01$ (** or ##), $p < 0.001$ (*** or ###) and $p < 0.0001$ (**** or ####) were considered statistically significant.

RESULTS AND DISCUSSION

Hemagglutinating activity and carbohydrate inhibition

The albumin fraction of the *Mucuna pruriens* seeds proved capable of agglutinate rabbit erythrocytes both treated and not treated with proteolytic enzymes (4 UH/ml), but no human erythrocytes agglutination was observed under the same conditions. Mo and Goldstein (1994) observed that the lectin obtained from seeds of *M. derringiana* was capable of agglutinate rabbit erythrocytes treated with trypsin. Obochi et al (2007), when characterizing a lectin present in the seeds of *M. sloanei* observed agglutinating activity using native human ABO system erythrocytes; the same lectin later purified by Teixeira-Sá (2009), who reported human and rabbit erythrocytes' agglutination, both treated and not treated with bromelain. Opposing our findings on the hemagglutinating

activity, Udedibie and Carlini (1998) reported absence of lectins in Brazilian *M. prurensis* seeds tested using pig, human and rabbit erythrocytes.

The detection of lectins in *M. pruriens* seeds seems to be a highly variable factor, being a result of determinant ecological and climatic conditions, as observed on Brazilian and Nigerian *M. pruriens* seeds var. *Utilis* (Udedibie and Carlini, 1998). The stable tropical climate in India seems to be favourable to a better expression of lectins in *Mucuna* seeds (Siddhuraju et al., 2000, 1996), contrasting with the diverse Brazilian climate range, its large territory and geographical physiognomy variation cause significant climatic variation throughout the year, not contributing to an ideal expression of these proteins.

The lectin's specificity to carbohydrates reported that only the λ-carrageenan was capable of inhibititig the hemagglutinating activity at the concentration of 39.06 µg/ml. Other glycoproteins and simple-sugar carbohydrates tested could not inhibit the MPLEC activity even at the maximum concentration used (2500 µg/ml for glycoproteins and 250 nM for simple sugars). The results obtained differ from the observed on *M. sloanei* and *M. derringiana* seeds' lectins, both presenting specificity to D-galactose and its derivations (Mo and Goldstein, 1994; Teixeira-Sá et al., 2009). λ-Carrageenan is a sulfated polysaccharide extracted from sea algae, normally constituted by esters of potassium, sodium, calcium, magnesium and ammonium sulfate and repeated units of β-D-galactose-2-sulfate-(1→4)-α-D-galactose-2,6-disulfate. Although reports of the lectins' specificity to sulphated carbohydrates are scarce, Toda and collaborators (1981) had already observed that *Solanum tuberosum* and *Triticum vulgaris* lectins (WGA) are capable to interact with keratan sulfate, a sulfated glycosaminoglycan constituted by repeated units of (1→3)-β-D-galactosyl-(1→4)-β-D-N-acetylglucosamine-6-sulfate.

Due to the probable existence of carrageenan on cell membranes, the MPLEC hemagglutinant activity's inhibition probably takes place indirectly, through the action of the galactose residuals, hence presenting similarity to the carbohydrate linking of other lectins previously purified. The sulfate groups and the molecule size appear to have great importance on the lectin interaction, as MPLEC did not link to a sepharose CL 4B resin (a galactose polymer – data not demonstrated) nor had its hemagglutinating activity inhibited on the presence of D-galactose. Another unusual fact was observed on the lectins of *S. tuberosum* and *T. vulgaris* that demonstrated an enhancing on their inhibition sensibility when the sulfate groups of keratan were removed (Toda et al., 1981).

We highlight the importance of this characteristic observed in MPLEC, as the literature does not report any other vegetal lectin capable of interaction with the carrageenan glycoprotein. Some lectins may display low affinity to monosaccharides or disaccharides due to their active site's spatial conformation, that demand in some cases more complex molecules such as glycoproteins, which favour an enhanced chemical interaction between the lectin and the carbohydrate (Gabius et al., 2011). The proposition is observed in many lectins isolated from legumes such as the agglutinins from *Phaseolus coccineus* (Chen et al., 2009), *Acacia constricta* and *Phaseolus vulgaris* (Guzman-Partida et al., 2004).

Lectin purification and PAGE

We submitted the albumin protein fraction to molecular exclusion chromatography, resulting in the elution of three distinct protein peaks (PI-ME, PII-ME, PIII-ME); only PI-ME displayed hemagglutination activity on rabbit erythrocytes (Figure 1A). The eluted active peak from the molecular exclusion column went through ionic exchange column, obtaining a peak of protein that was not retained by the matrix and had no agglutinant activity (PI-IE) and a peak of protein retained by the matrix and with agglutinant activity (PII-IE). The latter was eluted in a concentration of 0.85 M of NaCl (Figure 1B). The protein content and specific activity of the purification process can be seen in Table 1. It can be seen that the purification process resulted in a yield of 80% and an isolation of 32.98 times compared to the total protein of saline extract. These results indicate that the process performed is highly viable in both financial parameters as in final yield. The electrophoretic profile analysis for the active peak obtained by the ionic exchange on PAGE revealed a protein with approximate molecular weight of 60.0 kDa (at native conditions) and a protein pattern apparently pure compared to the albumin fraction of its origin seeds (Figure 2A and B).

The result presents similarity to what was observed on the lectin obtained from *M. sloanei*, a protein with ~65.6 kDa molecular weight estimated by molecular filtration chromatography on Superdex 75-HR column (at native conditions) and two bands by SDS-PAGE (36 and 34 kDa) (Teixeira-Sá et al., 2009). There was low similarity with the high molecular weight of the *M. derringiana* lectin, that presents 90.0 kDa (Mo and Goldstein, 1994), in native conditions. The results presented reflect important characteristics of the *Mucuna* lectins, displaying high molecular weight, an atypical characteristic for legume lectins, as well as (direct or indirect) affinity for galactose and its structural derivations, hence confirming what was proposed by several authors on the homology of lectins from vegetal species of the same genus and family (Sharon and Lis, 1990). The homology presented in lectins is observed and confirmed by the lectins from the subtribe Diocleinae, in which studies have already been carried out, especially on species of *Canavalia, Dioclea* and *Cratylia*. These lectins present specificity for the monosaccharides glycose and mannose, and possess high level of similarity in their amino acid sequences and three-dimensional structure (Loris et al.,

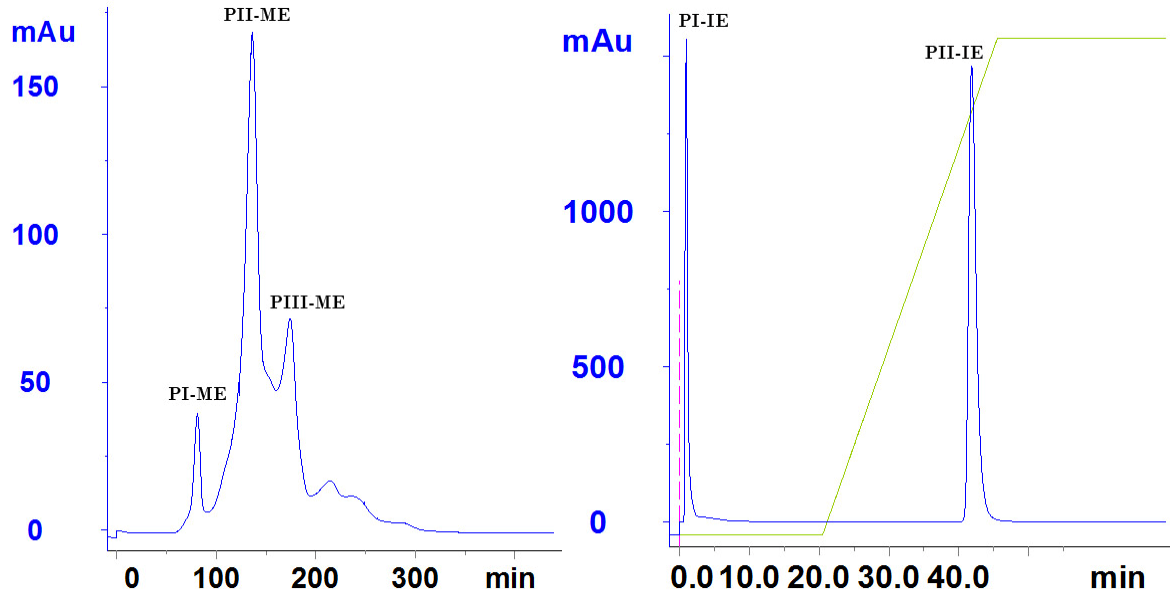

Figure 1. (A) Chromatographic pattern for the albumin fraction of the *Mucuna pruriens* seeds in a molecular exclusion column Sephacryl S-200 HR HiPrep 26/60 with volume 320 mL and constant flow rate of 1.3 mL/min linked to an AKTAprime plus system. The column was equilibrated and eluted with NaCl 0.15 M. **(B)** Chromatographic pattern for the active peak obtained by the molecular exclusion re-chromatographed in a 1 mL DEAE Sephacel HiPrep FF 16/10 ionic exchange column linked to an AKTAprime plus system. The column was equilibrated with Tris 0.025 M pH 7.6 and eluted with the same buffer containing NaCl (0-1 M). The chromatogram is expressed in absorbance *versus* elution time (minutes). Blue, green and pink lines indicate the absorbance, NaCl concentration and moment of sample injection, respectively.

Table 1. Purification of the lectin from *Mucuna pruriens* seeds.

Parameter	mg P/ml*	Specific activity	Yield (%)	Fold purification
Total extract	38.34	0.05215	100	1
Albumin fraction	3.7	1.0810	20	20.72
PI ME	7.35	1.0884	40	20.86
PII IE	9.3	1.7204	80	32.98

*mgP/ml represents the amount in milligrams of protein in 1 millilitre.

2004), yet, they have displayed different biological activities, differing as well on potency and efficiency for the same activities (Cavada et al., 2001; Moreno et al., 2004).

Hemolytic activity on human erythrocytes

The *M. pruriens* lectins did not present hemolytic activity on human erythrocytes. The hemolytic levels observed were lower than the ones demonstrated by saline (negative control), indicating that the lectin probably protected all types of erythrocytes from the natural hemolysis suffered during the experiment (Figure 3). The hemolytic activity profile for erythrocytes is measured based on studies by Rangel et al. (1997), where a substance is considered with low hemolytic effect when it causes the liberation of 0.0 to 40% of the haemoglobin compared to a substance with high hemolytic capacity (over 80% of hemoglobin liberation), such as Triton X. Similar results were found by Leite and collaborators (2012), who evaluated the hemolytic activity of a lectin-like protein present in *Clitoria fairchildiana* seeds, also registering very low hemolytic effects for human erythrocytes. In cases like those, it is necessary to evaluate the risk-and-benefit factor for potential pharmacological application. We highlight the importance of these results, as if MPLEC becomes a candidate to therapeutic use, it would only be useful if there were no significant damage to the biological system, especially fundamental structures, such as erythrocytes.

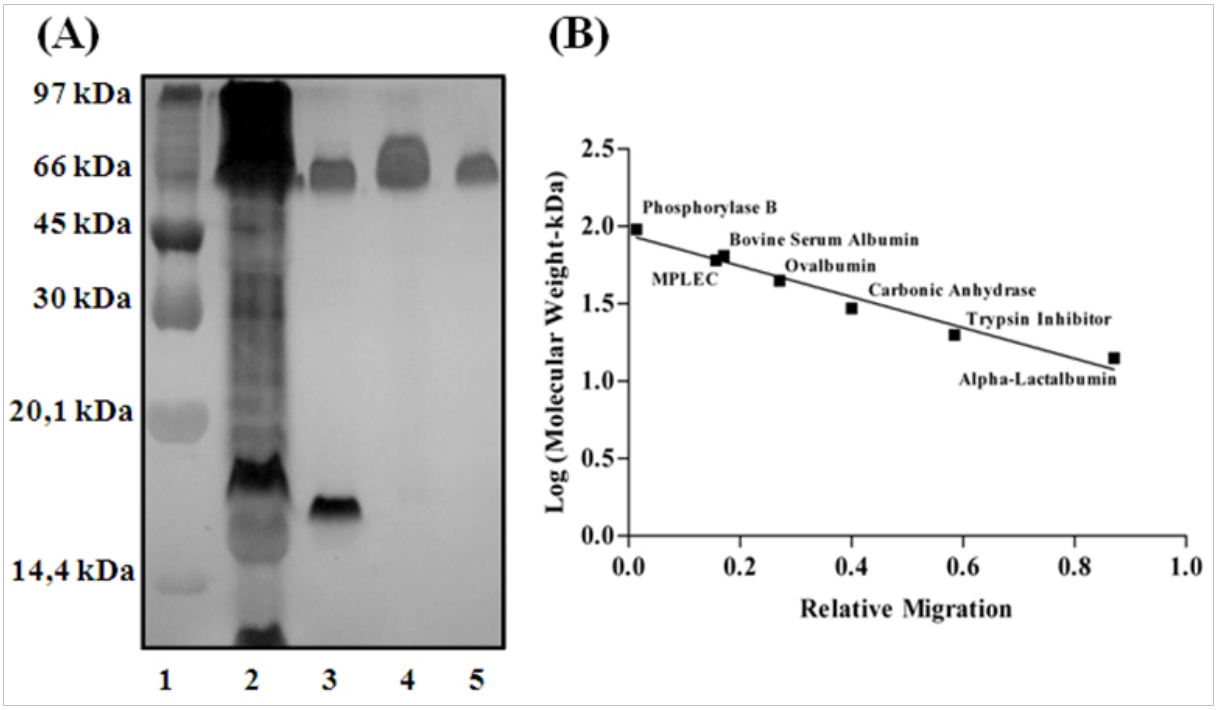

Figure 2. (A) PAGE **(Well 1)** Molecular mass patterns (top to bottom): Phosphorilase B (97.0 kDa), bovine serum albumin (66.0 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (30.0 kDa), trypsin inhibitor (20.1 kDa) and alpha-lactalbumin (14.4 kDa). **(Well 2)** *Mucuna prurensis* extract. **(Well 3)** Albumin fraction. **(Well 4)** Active peak from the Sephadex G-100 molecular exclusion column. **(Well 5)** Active peak from the DEAE-Sephacel ionic exchange column (MPLEC). **(B)** Molecular weight prospection by MPLEC migration observed on PAGE. Compared to known molecular weight markers.

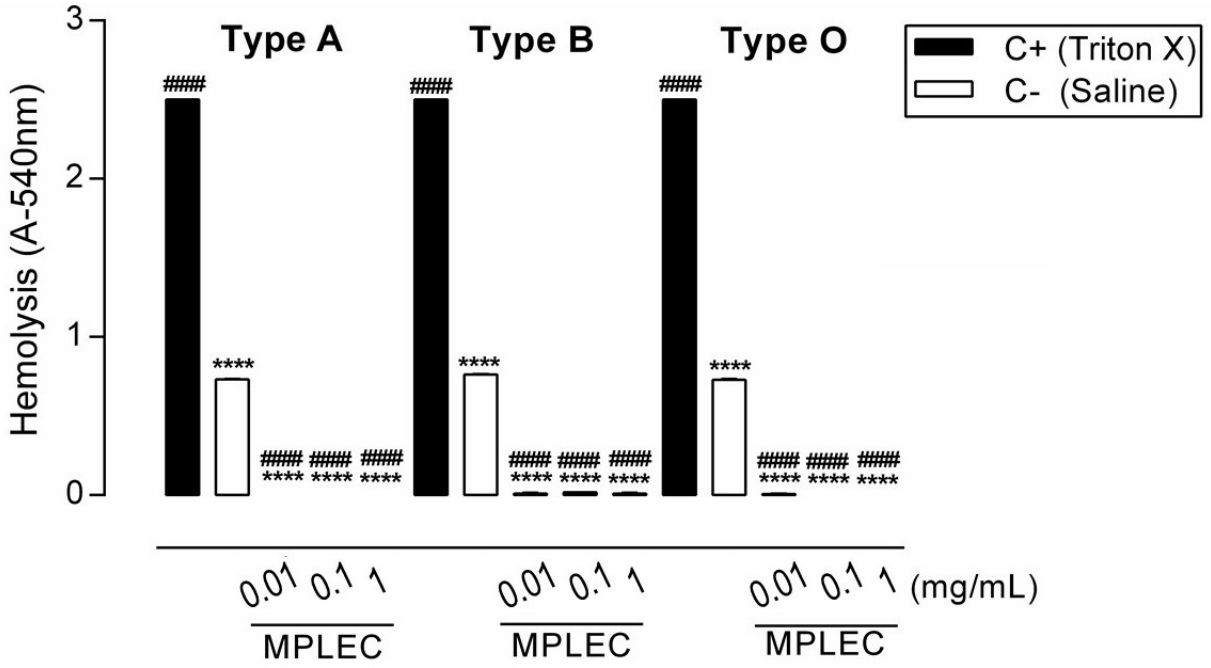

Figure 3. MPLEC hemolytic activity on human ABO erythrocytes. Results expressed in mean ± SEM of the hemolysis observed in three independent experiments *versus* the performed treatments. ****p<0.0001 compared to positive control (Triton X). ####p<0.0001 compared to negative control (saline) (ANOVA, Turkey's test).

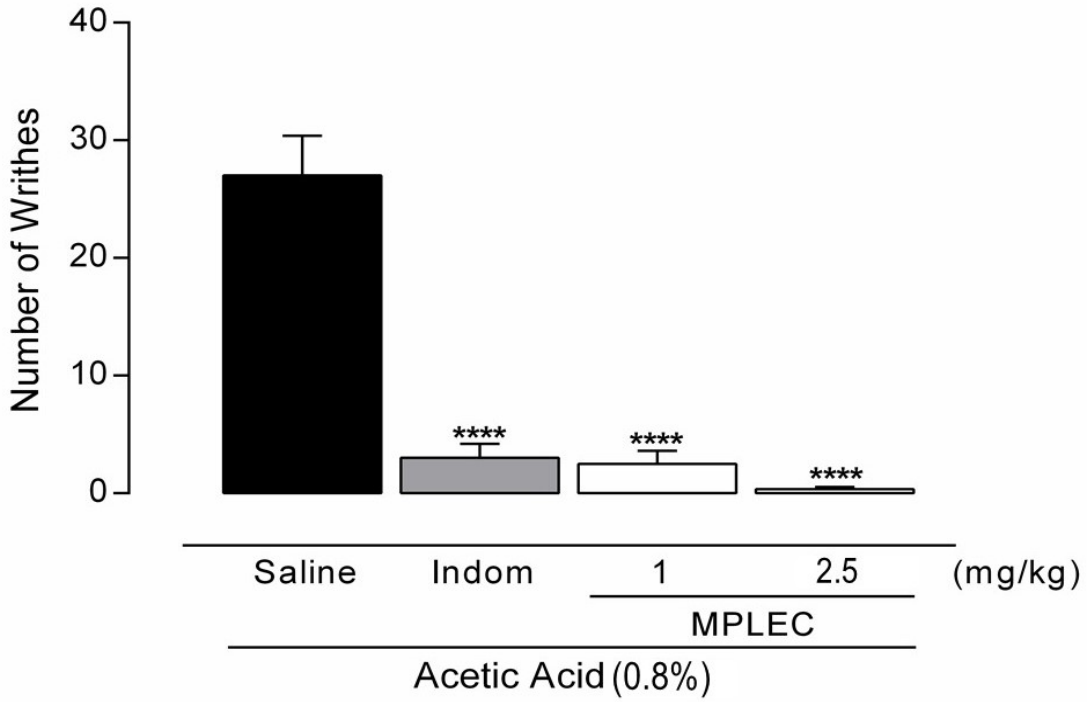

Figure 4. MPLEC reduced pain sensitivity on the acetic acid-induced abdominal contortions on mice. Mice received saline (0.1 mL/10g) or Indometacin (10 mg/Kg) or MPLEC (1 and 2.5 mg/kg). The analgesia was measured during 30 minutes after administrating the acetic acid and was expressed by the number of contortions the animal displayed. Data expressed by mean ± SEM of seven animals per group.
****p<0.0001 compared to negative control (saline) (ANOVA, Turkey's test).

Antinociceptive activity

Acetic acid-induced abdominal contortion model

MPLEC administration by intraperitoneal application (1 and 2.5 mg/kg) 30 min before the acetic acid inhibited the animals' abdominal contortions (91 and 99% of contortion inhibition, respectively) (Figure 4). The acetic acid is a chemical agent known for its irritant action caused by the ion acetate provoking intracellular acidification, resulting in epithelial cell lesion (Zelitlin and Norris, 1983), inducing the secretion of mediators such as prostaglandins (Ikeda et al., 2001), that activate chemo-sensitive nociceptors causing peripheral inflammatory pain (Julius and Basbaum, 2001). Although it is a test of low specificity, as many non-analgesic drugs (antihistamines, Central Nervous System stimulants, serotoninergic antagonists, neuroleptics and others) may also inhibit the acetic acid-induced contortions (Rates and Barros, 1994), its use as a pharmacological screening procedure still constitutes one of the main models for analgesic activity identification due to its simplicity, execution haste and reduced costs.

The results obtained by the animal treatment with MPLEC displayed a remarkable pharmacological efficiency compared to the non-steroid anti-inflammatory Indometacin used as test control, as the concentration of 2.5 mg/kg of lectin practically abolished the animals' contortions. The referred experimental model has been widely applied to verify analgesic activity promoted by lectins present in vegetal species (Holanda et al., 2009) and algae (Silva et al., 2010), as observed in a lectin-like protein purified from *Clitoria fairchildiana* seeds, in which dose-dependent antinociceptive activity was also reported (Leite et al., 2012). The lectinic site seems to be involved significantly on the lectin-promoted antinociceptive activity, as reported in the analgesic agglutinins isolated from *C. boliviana* seeds and the red algae *Pterocladiella capilacea*, in which previous association of these lectins with their specific carbohydrates (glycose and mucin) blocked the protein from exert its function against the painful stimulus, resulting in no contortion inhibition (Figueiredo et al., 2009; Silva et al., 2010).

Formalin method

Systemic administration of MPLEC (1 and 2.5 mg/kg) 30 min before the formalin demonstrated significant antinociceptive effect on the foot-licking timespan only at the second lectin concentration tested (2.5 mg/kg), both on initial phase (51% inhibition) and late phase (77% inhibition). As expected, the opioid morphine (5 mg/kg) reduced the nociception significantly in both phases (51% on phase 1, 88% on phase 2), the activity being completely

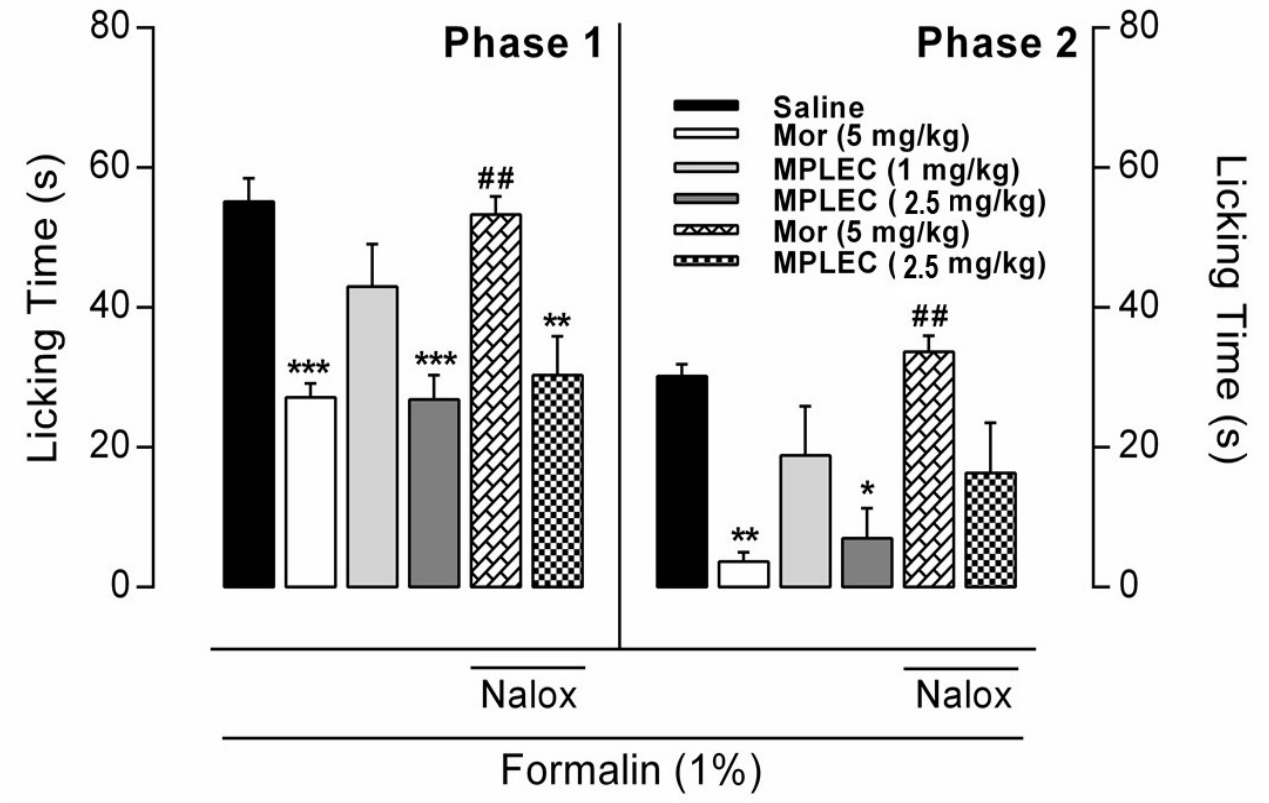

Figure 5. MPLEC reduces pain sensibility on formalin model in mice. Mice received saline (0.1 mL/10g), morphine (5 mg/Kg) or MPLEC (1 and 2.5 mg/Kg). Analgesia was measured on the first five minutes (phase 1, neurogenic), and the 20 following minutes (phase 2, inflammatory) after formalin administration and was expressed by the animal's time licking the injected foot (s). Naxolone (2 mg/Kg) was applied to revert the analgesia and verify the role of opioid receptors on the activity. Data expressed by mean ± SEM of seven animals per group.
*p<0.05, **p<0.01, ***p<0.001 compared to negative control (saline). ##p<0.01 compared to positive control (morphine) (ANOVA, Turkey's test).

reversed with the previous administration of the antagonist opioid receptor naloxone (2 mg/kg). No antinociceptive activity reversion observed at the test's initial phase with previous naxolone administration on the active lectin concentration, but at the late phase partial reversion was observed, yet not significant (Figure 5). The formalin test is considered a more specific model to analgesic drug test, as analgesic drugs present different responses on foot licking timing according to the test phase: the first five minutes (neurogenic phase) are related to drugs that act on opioid receptors system, occurring direct nociceptor stimulation with secretion of substance P, being a Central Nervous System stimulus. The following 20 minutes (inflammatory phase) are linked to anti-inflammatory action (Tjølsen et al., 1992) when chemical mediators, such as amino acids (Tjølsen and Hole, 1997), PGE2 (Malmberg and Yaksh, 1995), histamine (Gaertner et al., 1999), and others are involved, constituting a peripheral nervous system stimulus. It is known that opioid drugs inhibit both phases similarly, and the anti-inflammatories act mainly in the second phase (Hunskaar and Hole, 1987).

In our study, we could observe inhibitory stimuli in both phases in which the bigger concentration (2.5 mg/kg) was considered active. Probably MPLEC may be acting in a mixed action, but with a stronger anti-inflammatory characteristic as the inhibition was more efficient at the second phase of the test; suggesting that lectin can act both in peripheral and central sites. Such statement is confirmed by the fact that the antinociceptive activity is not completely reverted by naxolone, indicating that the compound is of a different receptor and not the classic opioid receptors. Similar results were observed by Vanderlei and collaborators (2010) on the antinociceptive effects of the lectin extracted from *C. cupressoides* where the antinociceptive activity was also only partially blocked by naxolone, suggesting a mainly peripheral effect. The effect of lectins on painful stimuli has presented wide variation of stimulated receptors. The algae lectins appear to act mainly on the peripheral nervous system (Bitencourt et al., 2008; Vanderlei et al., 2010), and the legume lectins appear to act at the Central Nervous System (de Freitas Pires et al., 2013; Figueiredo et al., 2009), although it is not a universal claim.

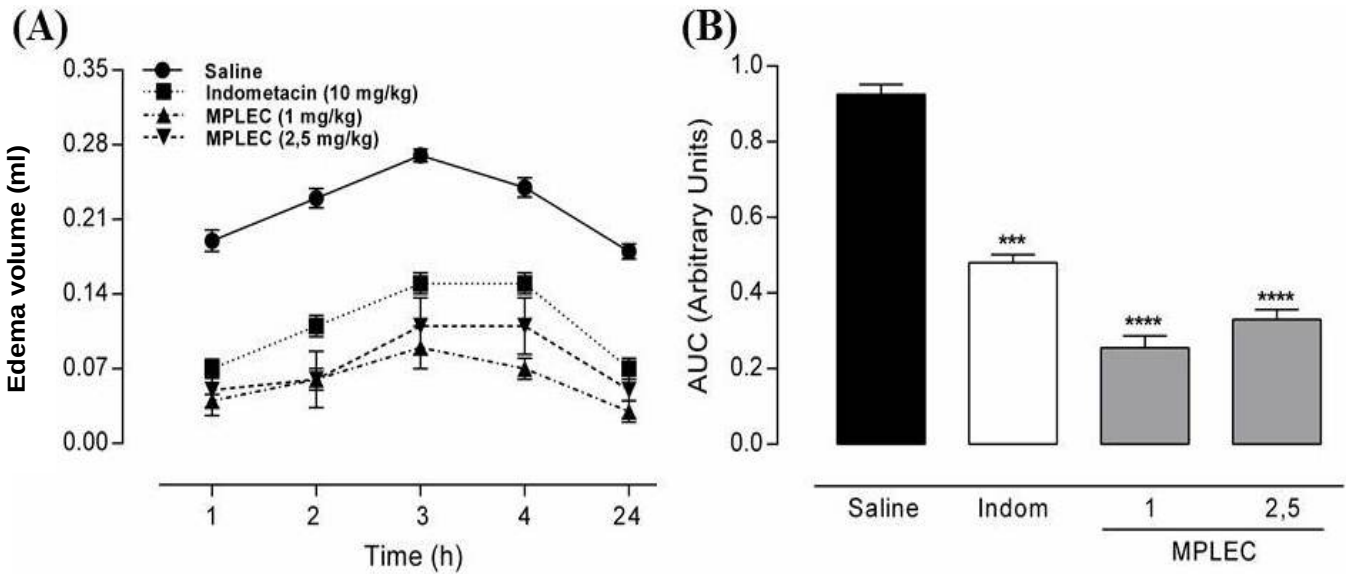

Figure 6. MPLEC reduces carrageenan-induced edema on feet. The mice received saline (0.1 mL/10g), Indometacin (10 mg/Kg) or MPLEC (1 or 2.5 mg/Kg). The edemas were measured 1, 2, 3, 4 and 24 hours after the carrageenan induced inflammation and was expressed as the increase of foot volume (mL) (A) or the area under the curve in arbitrary units (B).
Data expressed as mean ± SEM of seven animals in each group. ***p<0.001, ****p<0.0001 when compared to the negative control (saline) (ANOVA, Turkey's test).

One class of receptor for analgesic exogenous substances, with ongoing studies, is the opioid-like receptors (ORL-1). These receptors are linked to a G protein and present connection with nociception already proven (Higgins et al., 2001; Reinscheid et al., 1995). It is not present naxolone-promoted analgesic action reversion might act directly on ORL-1 receptors; yet, more studies must be held on their specificity, applying drugs that antagonize the activity such as the ones that act on the ATP-dependent potassium channels (glibenclamide), since it is known that the ORL-1 receptors are sensitive to those (Armstead, 1999). Hence, if verified the MPLEC activity on this class of receptors, a new target for anesthetic and analgesic drugs is discovered.

Anti-inflammatory activity

The carrageenan caused intense edema that peaked within 3 h on the negative control group (saline) (0.27 ± 0.006) after the administration. The animals treated with MPLEC (1 and 2.5 mg/kg) by intraperitoneal administration 30 min before the carrageenan significantly reduced the edema occurrence when compared with the saline treatment. As expected, the treatment with the anti-inflammatory Indometacin (10 mg/kg), the positive control, also reduced the animals' edemas (Figure 6A). After the derivation of the points obtained during the experiment in area over the curve distribution with arbitrary units, it was possible to known that these receptors are involved directly on the nociceptive stimuli due to the action of specific antagonists that cause the analgesia, but their action is not blocked by opioid receptor antagonists such as naxolone (Byford et al., 2007). The substances that do determine the percentage of edema reduction observed. We registered a diminution of 72 and 64% for MPLEC 1 and 2.5 mg/kg, respectively, and 48% for the positive control Indometacin 10 mg/kg, resulting in MPLEC displaying a remarkable pharmacological response compared to the control group, taking into account the dosage applied on the test (Figure 6B). Despite the MPLEC be inhibited by this flogistic agent, a phenomenon occurs so that a higher concentration of carrageenan is necessary or equal to 39.06 mg/ml. The concentration of carrageenan paw edema used in the test was 1% w/v, and the injected volume of 40 µl of the agent in the paw of the animal. These data gave us a value of concentration of 0.0004 µg/ml which is not enough to connect the lectin, and thus block its effect resulting in an anti-inflammatory false positive effect. Thus, the end of the bioavailable fraction agent is capable of causing its pro-inflammatory effect, but is not able to inhibit the lectin injected in doses of 1 and 2.5 mg/kg.

The carrageenan is a polysaccharide extracted from algae and is considered as one of the principal chemical agents of inflammatory processes, with wide application in pharmacological screening tests that evaluate potential anti-inflammatory activity (Hajhashemi et al., 2010; Sadeghi et al., 2011; Whiteley and Dalrymple, 1998). The edema and inflammation caused by carrageenan occur in

three distinct phases in which diverse chemical mediators are involved: phase one presents histamine and serotonin liberation, phase two presents cytokine liberation and phase three display the action of prostaglandins (Lo et al., 1982). As presupposed by the antinociceptice activity test, MPLEC has anti-inflammatory activity, with antiedematogenic properties.

Many physiopathology mechanisms are involved in lectin-mediated anti-inflammatory process. They can inhibit neutrophil migration to the peritoneal cavity and their rolling and vascular adhesion (Mayadas and Cullere, 2005), as well as alter the pro-inflammatory cytokines production (Nunes et al., 2009). More studies are needed to clarify the probable mechanism in which MPLEC exert its function; although many legume lectins that present proven antiedematogenic activity already have their functional pathways partially known, such as the lectin-like protein from *C. fairchildiana* seeds and the lectin from *C. grandiflora*, that are capable of inhibiting neutrophil migration and alter the vascular permeability *in vivo* (Leite et al., 2012; Nunes et al., 2009).

Conclusion

A lectin from Brazilian *M. pruriens* seeds was isolated with 60 kDa mass, capable of agglutinating both native and enzymatically treated rabbit erythrocytes, with its activity inhibited by carrageenan. The lectin possess antinociceptive effect by mixed mechanism, anti-inflammatory and antihemolytic action. More studies are necessary to evaluate its *in vivo* biological action mechanism and three-dimensional structure.

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Conflict of interest

Authors have not declared any conflict of interest.

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Full Length Research Paper

Essential oil of *Cymbopogon flexuosus*, *Vernonia polyanthes* and potassium phosphite in control of bean anthracnose

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This study evaluated essential oils of *Cymbopogon flexuosus* (EOC), *Vernonia polyanthes* (EOV), and potassium phosphite (PP) in control of bean anthracnose caused by *Colletotrichum lindemuthianum*. This study assessed mycelial growth and conidial germination *in vitro*, scanning electron microscopy (SEM), and enzymes peroxidase (POX) and phenylalanine ammonia-lyase (PAL). EOC and EOV in doses 1,450 and 1,320 µL L^{-1} reduced disease severity by 57.2 and 37.6%, respectively. Major components identified in EOC were geraniol 46.8% and nerol 33.4%, and germacrene-D (42.2%) and bicyclogermacrene (17.2%) in EOV. SEM images showed that PP, EOC and EOV reduced mycelial growth and emission of germ tubes. PP and EOC increased POX and PAL rates in bean tissue. Bean anthracnose was controlled by direct antifungal activity of PP and EOC and induction of defense enzymes

Key words: Alternative disease control, *Colletotrichum lindemuthianum*, resistance induction, scanning electron microscopy, *Phaseolus vulgaris*.

INTRODUCTION

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc & Magnus) is a major destructive disease of common bean (*Phaseolus vulgaris* L.). The seed borne fungi has high pathogenic variability with over 100 described strains (Campa et al., 2011). In Brazil, infected seeds may cause up to 100% losses in bean yield under favorable climatic conditions (Damasceno et al., 2007).

The most economically viable and effective strategy for disease control is by using bean cultivars resistant to fungi (Ishikawa et al., 2008). However, this kind of management is difficult to implement due to the high pathogenic variability of *C. lindemuthianum* (Ishikawa et al., 2012). Consequently, synthetic fungicides are widely used by farmers to control bean anthracnose

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(Gillard et al., 2012). Short term use of these products effectively helps the farmer achieve high yields. Thus, several research groups have sought cheaper and low-toxicity alternatives for controlling fungi pathogenic to plants. Essential oils (EOs) and potassium phosphite (PP) are potentially useful both for their direct toxicity for fungi, which inhibit mycelial growth and spore germination, as for induction of phytoalexins, which indicate the presence of elicitor compounds (Pereira et al., 2012).

This study assessed essential oils of *Cymbopogon flexuosus* (EOC), *Vernonia polyanthes* (EOV), and potassium phosphite (PP) to control of bean anthracnose caused by *C. lindemuthianum*.

METHODOLOGY

Essential oil extraction

Leaves of *C. flexuosus* and *V. polyanthes* were collected in the season of winter on the month August in the city of Lavras. Essential oils were extracted from 1000 g fresh leaves through steam distillation for 90 min, using a Marconi MA480 stainless steel distiller. The aqueous phase was extracted with dichloromethane (3 × 25 ml). The organic phase was dried with anhydrous magnesium sulfate, filtered and the solvent evaporated until dryness.

Essential oil analysis

Analysis of chemical composition was performed according to Rosado et al. (2013). Oil quantitative analysis was performed using gas-phase chromatography coupled to a hydrogen flame ionization detector (GC-FID) on Agilent® 7890. A system equipped with HP-5 fused in the electron impact ionization mode (70 eV), injector split, capillary colum HP-5 (30 m × 0.25 mm × 0.25 µm). Temperature: injector = 220°C, collum = 60°C for 1.5 min, ramp = 3°C for min. Carrier gas He = 1 ml min^{-1}.

Retention indices (RI) have been obtained according to the method of Van den Dool and Dec Kratz (1963).

Analysis of mycelial growth inhibition in *C. lindemuthianum*

Pure culture of *C. lindemuthianum,* strain 65, isolate LV 175 was provided by the Laboratory of Plant Resistance, Department of Biology, UFLA. The single spore culture was obtained. In inhibition test of mycelial growth, the treatments were EOC and EOV at concentrations of 125, 250, 500, 1000, and 2000 ml L^{-1}, PP at a dose of 5 ml L^{-1} and trifloxystrobin + fungicide at a dose of 0.75 ml L^{-1}. All treatments were added to potato dextrose Agar (PDA) medium containing Tween 20 (0.1%). A treatment containing Tween 20 (0.1%) was used to isolate its effect and treatment with sterile distilled water as negative control. After solidification, mycelial disks (8 mm) of *C. lindemuthianum* were placed in the center of petri dishes (90 mm) with the treatments described earlier and incubated at 25°C under photoperiod of 12 h.

Evaluations were performed daily until fungal colonies covered 2 thirds of the medium surface. Percentage of growth inhibition (PGI) was calculated using the formula: PGI = [(diameter of control - diameter of treatment) / diameter of control] × 100 for each treatment as compared to the control. The experiment was conducted in completely randomized design with six replicates, each plot consisting of a petri dish.

Evaluation of conidial germination

In this study, suspensions of *C. lindemuthianum* conidia growing at PDA medium in test tubes were used. Sterile distilled water (10 ml) was added into the test tubes and they were agitated. The suspension obtained was filtered with sterile gauze and its concentration was adjusted to 1.15×10^5 conidia ml^{-1} in a Neubauer chamber. One milliliter spore suspension and 1 ml of the treatments described earlier were spread over the surface of petri dishes (60 mm) with the water ágar medium at 2% with handles Drigalski. Then the plates were incubated at 25°C under a 12-h photoperiod. After 24 h, lactoglycerol was used for stopping the germination process.

Percentage of growth inhibition (PGI) was evaluated by counting the number of germinated and non-germinated conidia. PGI was obtained using the formula: PGI = [(number of germinated conidia control - number of conidia in the treatment) / number of conidia in the control] × 100 for each treatment as compared to the control. The experiment was conducted in completely randomized design with six replicates, each plot consisting of a petri dish.

Evaluation of treatments in anthracnose control

For evaluation of treatments in anthracnose control bean seedlings of cultivar Pearl was used. Twenty one days after sowing, the treatments were performed. 48 h after application, the plants were sprayed with a suspension of inoculum of 1×10^6 and incubated in a humid chamber for 14 h. The experiment was conducted in a randomized block design with four replications. Each plot consisted of two pots containing three plants each.

Anthracnose severity was assessed every seven days through a rating scale proposed by Godoy et al. (2006). The area under the disease progress curve (AUDPC) was calculated for each treatment, according to Shaner and Finney (1977).

Enzymatic activity of peroxidase (POX) and phenylalanine ammonia lyase (PAL)

To determine the enzymatic activity of peroxidase (POX) and phenylalanine ammonia-lyase (PAL), the most promising treatments were selected. Distilled water was used as negative control. Bean plants at the stage V3/V4 were sprayed after 7 days and were inoculated as previously described. The experiment was a randomized complete block design with three replications and the portion composed of three plants.

Bean leaf samples were collected on days 1, 3, 6, 8, 10 and 13 after spraying treatments. The samples were stored at -80°C for later analysis.

To determine enzymatic 0.2 g of leaf tissue with 1% polyvinylpyrrolidone and liquid N_2 were ground in a mortar. It was homogenized in 1.3 ml of 100 mM potassium phosphate buffer (pH 7.0) containing 1 mM phenylmethylsulfonyl fluoride, and was centrifuged at 11,000 ×g for 30 min at 4°C and the supernatant was used for enzymatic determination.

The POX activity was determined by oxidation of guaiacol according to the method of Kar and Mishra (1976). Ninety microliters of potassium phosphate buffer 100 mM (pH 7.0), 30 µl of guaiacol 40 mM and 25 µl of H_2O_2 125 mM were added to 10½ extract. Absorbance was measured at 420 nm in ELISA reader every 10 s for 2 min after adding the sample to the mixture. Molar extinction coefficient of 1.24 mM cm^{-1} was used to calculate the POX activity (Maehly and Chance, 1955), which was expressed in mM purpurogallin produced min^{-1} mg^{-1} protein.

The PAL activity was measured by adding 40 µ to a mixture containing 110 µl of 100 mM Tris-HCl (pH 8.8) and 50 µl of 100 mM L-phenylalanine. The reaction mixture was incubated in the ELISA

reader at 37°C for 10 min. The absorbance of derivatives of trans-cinnamic acid was measured in a spectrophotometer at 280 nm and the molar extinction coefficient of 5000 was used mM^{-1} cm^{-1} (Zucker, 1965) for calculating the PAL activity, which was expressed in mM min^{-1}-mg protein. The protein concentration in each sample was determined according to the colorimetric method described by Bradford (1976).

Analysis in scanning electron microscope

Pearl bean cultivar at V3/V4 stage was sprayed with the most promising treatments: OEV and OEC 1000 µl L^{-1} and PP 5 µl L^{-1}. As a negative control, sterile distilled water was sprayed. Two days after application, seven of the third trifoliate leaves were collected and packaged in plastic trays with the bottom of the tray covered with foam rubber and two sheets germitest wetted with distilled water.

Points were marked and 25 µl of suspension with 1.5×10^3 spores of *C. lindemuthianum* ml^{-1} was deposited. After inoculation, the trays were covered with clear plastic and placed in a growth chamber at 25°C until the end of collection.

Samples were collected after 4, 8, 16, and 48 h of inoculation, using 5 mm circular cuts with a scalpel inoculated into each point. The samples were placed Karnovsky's fixative at pH 7.2 and stored at 4°C for 24 h. After this period, the samples were placed in 0.05 M cacodylate tampon and washed three times for 10 min and transferred to osmium tetroxide and 1.0% water for 1 h, washed three times with distilled water.

Then the samples were dehydrated in acetone series (25, 50, 70, 90, and 100% three times) and brought to the critical point dryer Balzers CPD 030 for replacing acetone by CO_2. The samples were mounted on stubs and coated with gold using the Balzers SCD 050 evaporator for observation in a scanning electron microscope LEO EVO 40. The images were digitally generated 20 kV to a 10 mm working distance, then the images were worked in Corel Draw Photo Paint 12.

Statistical analysis

Data were subjected to analysis of variance, and mean values were compared by the Scott Knott test at 5% probability for qualitative factors, while regression analysis was applied for quantitative factors.

RESULTS

Yields and chemical composition

Chemical composition of essential oils of *C. flexuosus* and *V. polyanthes* are as shown in Table 1. Oil yields were 1.27 and 0.02% (v/w), respectively for *C. flexuosus* and *V. polyanthes*.

Twenty compounds were identified in the essential oil extracted from *C. flexuosus* leaves, representing 97.7% of total constituents. Twenty two compounds were identified in *V. polyanthes* leaf essential oil, representing 97.6% of the total constituents.

The composition of *C. flexuosus* essential oil is much simpler and mainly composed by monoterpenes (96.4%) in contrast to *V. polyanthes* essential oil, which is primarily composed of sesquiterpenes (94.3%).

Evaluation of mycelial growth inhibition and conidial germination in *C. lindemuthianum*

Essential oils of *C. flexuosus* and *V. polyanthes* inhibited mycelial growth and conidial germination in *C. lindemuthianum*. Oil of *C. flexuosus* was more efficient, and reduced colony diameter in *C. lindemuthianum* starting from the lowest concentration (125 µl L^{-1}). Total suppression of fungus mycelial growth occurred in concentrations higher than 615 $µl^{-1}$ of EOC (Figure 1). The concentration capable of inhibiting mycelial growth was 50% in 162 µl L^{-1} and 1918 µl L^{-1} in EOC and EOV, respectively.

EOC was more efficient for percentage of conidia germination inhibition, and concentration of 689 µl L^{-1} totally inhibited *C. lindemuthianum* germination (Figure 1C). The concentration capable of inhibiting at least 50% conidial germination was estimated in 220 and 850 µl L^{-1} in EOC and EOV, respectively.

Table 2 compares the effect of essential oil (EO) doses, showing higher antifungal activity with potassium phosphite (PP), fungicide (F), and EOC at 1000 µl L^{-1}. The highest antifungal activity occurred with F and EOC, which completely inhibited mycelial growth and fungal germination.

Essential oils and potassium phosphite in control of bean anthracnose in greenhouse

In greenhouse, essential oils of *C. flexuosus* and *V. polyanthes* reduced the area under the curve of progress of anthracnose (AUCPA). EOC provided greater reduction (57.2%) as compared to the control in concentration 1450 µl L^{-1} whereas EOV provided 37.6% as compared to the control in concentration 1320 µl L^{-1} (Figure 2).

Treatments with potassium phosphite and fungicide reduced AUCPA by 60.4 and 62.1%, respectively, compared to control. There was no statistical difference between PP, F and EOC at 1000 µ L^{-1}.

Studies of germination and mycelial growth *in vivo* in *C. lindemuthianum*

Images of scanning electron microscopy showed no conidia germination in *C. lindemuthianum* in all treatments 4 h after inoculation. Samples collected 8 h after inoculation had no germination either, except for the treatment with distilled water. In this treatment, we observed conidia starting the germination process (Figure 3).

Bean leaves treated with distilled water 16 h after inoculation showed conidia in advanced stage of germination and mycelial growth on the surface of leaves. However, leaves treated with EOC and EOV showed low

Table 1. Relative concentration of constituents of essential oils from fresh leaves of *Cymbopogon flexuosus* and *V. polyanthes.*

Compound	RI[a]	*C. flexuosus*	*V. polyanthes*
α-Pinene (M)	830	-	0.24
β-Pinene (M)	874	-	2.06
Methyl-heptenone (OM)	883	1.01	-
Myrcene (M)	988	0.72	-
β-Terpinene (M)	985	-	0.11
Ocimene (M)	994	-	0.35
β-Ocimene (M)	1020	0.21	-
Linalol (OM)	1098	2.47	-
exo-Isocitral (OM)	1144	0.4	-
Citronelal (OM)	1152	0.21	-
Z-Isocitral (OM)	1164	1.42	-
Not identified m/z=152	1182	2.01	-
Estragole (OM)	1198	0.58	-
N-Decanal (OM)	1205	0.36	-
Neral (OM)	1243	33.40	-
Geraniol (OM)	1254	1.14	-
Geranial (OM)	1273	46.86	-
E-Dimethoxycitral (OM)	1338	-	0.58
Not identified m/z=168	1339	1.01	-
Not identified m/z=168	1375	1.45	-
Cyclosativene (S)	1377	-	2.34
Geranil acetate (OM)	1384	3.08	-
Cycloisosativene (S)	1385	-	1.74
Copaene (S)	1393	-	1.96
trans- Caryophyllene (S)	1420	0.39	-
β-Caryophyllene (S)	1421	-	13.57
Gurjunene (S)	1430	-	0.49
α-Humulene (S)	1455	-	7.84
cis-Cadina-1(6), 4-diene (S)	1462	-	1.96
Ar-Curcumene (S)	1479	0.31	-
Germacrene-D (S)	1484	-	42.18
δ-Curcumene (S)	1496	0.34	-
Bicyclogermacrene (S)	1498	-	17.23
Germacrene A (S)	1506	-	0.77
(E,E)-α-Farnesene (S)	1509	-	0.91
γ-Cadinene (S)	1515	-	0.42
δ-Cadinene (S)	1524	-	1.13
Spathulenol (OS)	1578	-	0.38
Caryophyllene oxide (OS)	1583	0.24	0.84
α-Muurolol (OS)	1642	-	0.22
α-Cadinol (OS)	1655	-	0.20
Total		97.69	97.62

[RI]Retention indices (13). [%]Compounds percentage. [a]Retention indices relative to *n*-alkanes (C_8-C_{20}) on the HP-5 MS Capillary column. M: Monoterpene; OM: oxygenated monoterpene; S: sesquiterpene; OS: oxygenated sesquiterpene.

conidia germination while the treatment with PP had no germination (Figure 3). Large differences were found 48 h after inoculation. Leaves treated with distilled water had clear mycelial growth and emission of numerous germ tubes near ribs in the abaxial surface. Conversely, leaves sprayed with essential oils showed low conidia germination

Table 2. Percentage of inhibition of mycelial growth (PIMG) and conidia germination (PICG) in *Colletotrichum lindemuthianum* subjected to different treatments with essential oils of *C. flexuosus* (EOC) and *V. polyanthes* (EOV), potassium phosphite (PP), fungicide (Trifloxistrobina + Tebuconazol) and control (H_20).

Treatment	PIMG	PICG
PP	100^{a}	100^{a}
EOC 1000 µl L^{-1}	100^{a}	99.13^{a}
Fungicide	76.08^{b}	93.3^{b}
EOV 2000 µl L^{-1}	50.21^{c}	50.1^{c}
Tween 20	0.64^{d}	0.4^{d}
Control (H_20)	0^{d}	0^{d}
CV (%)	5.04	4.52

Means followed by the same letter in the column do not differ statistically by Tukey test at 5% probability. Data were transformed to $\sqrt{x} + 1$.

Table 3. Activity of peroxidase mM min^{-1} mg^{-1} protein in bean plants with and without inoculation of *C. lindemuthianum* at stage V3/V4 collected at 8, 10 and 13 days after spraying with potassium phosphite (PP) 5 ml L^{-1}, essential oil of *C. flexuosus* (EOC) 1000 µl L^{-1} and distilled water (H_2O).

Treatment	Without inoculation			With inoculation		
	8^{NS}	10	13	8	10	13
PP	189.24^{a}	147.56^{a}	163.76^{a}	202.47^{aC}	326.8^{aB}	382.03^{aA}
EOC	118.14^{b}	134.43^{a}	150.87^{a}	192.92^{aB}	334.36^{aA}	315.21^{bA}
H_2O	150.29^{b}	140.93^{a}	131.1^{a}	145.06^{bC}	203.1^{aB}	311.43^{bA}
CV (%)	14.34	-	-	-	-	-

Means followed by the same letter, lowercase in columns and capital letters in lines do not differ by the Scott-Knott test at 5% probability. ^{NS}There was no statistical difference for the factor time of collection x treatments for analysis of non-inoculated plants (p = 0.1815).

and poorly developed mycelium. The treatment with PP had no conidia germination (Figure 3).

Biochemical analysis of induced resistance

For the enzymatic activities, only treatments with EOC and PP in concentration of 1000 µl L^{-1} and 5 ml L^{-1}, respectively were selected.

In inoculated plants, peroxidase (POX) activity was significantly higher in treatment with PP at 8 and 13 days after spraying differing from the control treatment. However, at day 10, there was no significant difference between treatments. Essential oil of *C. flexuosus* provided higher peroxidase activity at day 8 compared to the control. Increase of peroxidase at day 8 in treatments with PP and EOC helped control the pathogen, as inoculation was performed at 7 days after spraying. Plants recognized the fungus and increased the level of POX.

In non-inoculated plants at 8 days after spraying, only PP differed from the other treatments (Table 3). With respect to collection period, there was statistical difference in inoculated plants since PP showed higher content of POX at day 13 compared to days 8 and10. Plants sprayed with EOC had greater activity at 10 and 13 days after spraying. There was a significant difference between inoculated and non-inoculated plants, and plants challenged with fungus *C. lindemuthianum* showed higher levels of POX.

POX activity at 1, 3, 6, 8, 10 and 13 days after spraying in non-inoculated plants (Table 4) suggests that spraying PP had an active role in potentiating and anticipating enzyme activity. This result demonstrates the role of PP in plant defense, since there was difference from the control treatment at 1, 3, 6 and 8 days after spraying. With respect to EOC, there was significant difference compared to control at 1, 3, 6 and 8 days after spraying.

Regarding the activity of the enzyme phenylalanine ammonia lyase (PAL), there was significant difference. In

Table 4. Activity of peroxidase mM min^{-1} mg^{-1} protein in bean plants without *C. lindemuthianum* at stage V3/V4, collected at 1, 3, 6, 8, 10 e 13 days after spraying with potassium phosphite (PP) 5 ml L^{-1}, essential oil of *C. flexuosus* (OEC) 1000 µl L^{-1} and distilled water (H_2O).

Treatment	Days after spraying					
	1	3	6	8	10	13
PP	145.31^{aB}	203.6^{aA}	213.17^{aA}	189.24^{aA}	147.66^{aB}	163.76^{aB}
EOC	151.41^{aB}	193.87^{aA}	185.06^{bA}	118.14^{cB}	134.43^{aB}	150.87^{aB}
H_2O	107.04^{bB}	159.36^{bA}	158.7^{Ba}	150.29^{bA}	141.03^{aA}	131.1^{aB}
CV (%)	11.77	-	-	-	-	-

Means followed by the same letter, lowercase in columns and capital letters in lines do not differ by the Scott-Knott test at 5% probability.

Table 5. Activity of phenylalanine ammonia-lyase mMol min^{-1} mg $protein^{-1}$ in bean plants with and without inoculation of *C. lindemuthianum*, at V3/V4 stage, collected at 8, 10 and 13 days after spraying of potassium phosphite (PP) 5 ml L^{-1}, essential oil of *C. flexuosus* (EOC) 1000 µl L^{-1} and distilled water (H_2O).

Treatment	Without inoculation			With inoculation		
	8	10	13	8	10	13
PP	0.33^{Ab}	1.74^{aA}	0.22^{aB}	1.83^{aB}	2.45^{aA}	0.58^{aC}
EOC	0.25^{aA}	0.59^{bA}	0.55^{aA}	0.94^{bB}	1.98^{bA}	0.80^{aB}
H_2O	0.31^{aB}	0.79^{bA}	0.32^{aB}	0.45^{cA}	0.68^{cA}	0.17^{bC}
CV (%)	24.46	-	-	-	-	-

Means followed by the same letter, lowercase in columns and capital letters in lines do not differ by the Scott-Knott test at 5% probability.

Table 6. Activity of phenylalanine ammonia-lyase (PAL) in mMol min^{-1} mg $protein^{-1}$ in bean plants without inoculation of *C. lindemuthianum*, at V3/V4 stage, collected at 1, 3, 6, 8, 10 and 13 days after spraying of potassium phosphite (PP) 5 ml L^{-1}, essential oil of *C. flexuosus* (EOC) 1000 µl L^{-1} and distilled water (H_2O).

Treatment	Days after spraying					
	1	3	6	8	10	13
PP	1.31^{aC}	2.15^{bA}	1.69^{aB}	0.33^{aD}	1.74^{aB}	0.22^{bD}
EOC	0.73^{bC}	2.53^{aA}	1.44^{bB}	0.25^{aD}	0.59^{bC}	0.55^{aC}
H_2O	0.71^{bA}	0.98^{cA}	0.78^{cA}	0.31^{aB}	0.79^{bA}	0.32^{bB}
CV (%)	13.6	-	-	-	-	-

Means followed by the same letter, lowercase in columns and capital letters in lines do not differ by the Scott-Knott test at 5% probability.

plants inoculated and sprayed with PP, there was greater activity of PAL compared to plants sprayed with EOC and water to 8 and 10 d.a.p. (Table 5). Already at 13 d.a.p., plants sprayed with PP produced less PAL and not different from oil.

Considering PAL activity in non-inoculated plants at 1, 3, 6, 8, 10 and 13 days after spraying, PP was superior to other treatments at days 1, 6 and 10 (Table 6). Plants sprayed with EOC had greater PAL activity at days 3 and 13. There was no significant difference between treatments at day 8. Regarding time of collection, plants sprayed with PP and EOC showed higher PAL activity at day 3. The control treatment had increased PAL production at days 1, 3, 6 and 10.

DISCUSSION

The constituents of *C. flexuoso* in greater quantity were geranial (46.9%) and neral (33.4%), called citral, and

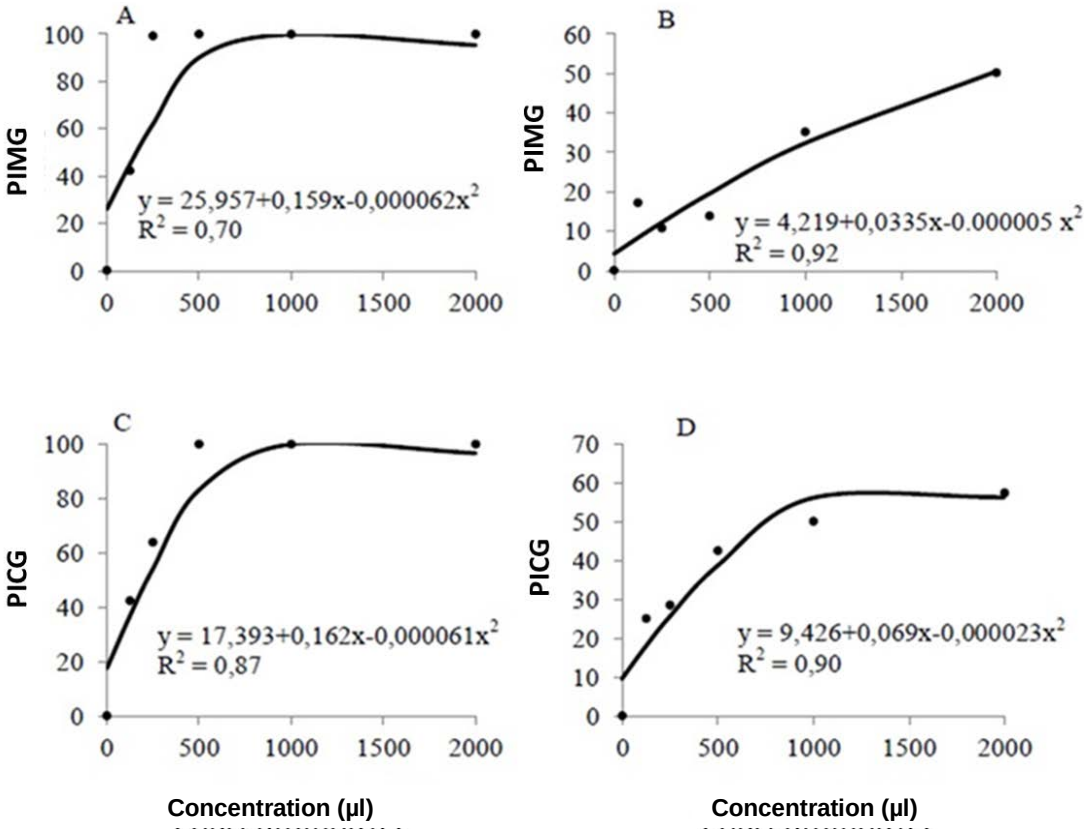

Figure 1. Percentage of inhibition of mycelial growth (PIMG) and conidial germination (PICG) in *C. lindemuthianum* at different concentrations (0, 125, 250, 500, 1000, and 2000 µL L^{-1}) of essential oils of *C. flexuosus* (A and C) and *V. polyanthes* (B and D). Data were transformed to √x + 1.

comprised 80.3% of the essential oil. These results are in agreement with the literature (Adukwu et al., 2012).

The constituents of *V. polyanthes* is mainly composed by germacrene D (42.2%), bicyclogermacrene (17.2%), β-caryophyllene (13.6%) and α-humulene (7.8%), representing 80.8% of total oil. These results are in agreement with the literature (Maia et al., 2010).

Fungicide and fungistatic effect were found when mycelium discs with 100% PIMG were taken from the culture medium at the end of growth analysis and subcultured to PDA plates. Only PP had fungistatic activity, whereas in the other treatments, the effect was fungicidal. This probably resulted from the high volatility of *C. flexuosus* essential oil, which killed fungal cells in the upper part of the mycelium disc that comes not in contact with culture medium.

Anaruma et al. (2010) determined the activity of 28 essential oils from medicinal plants against *Colletotrichum gloeosporioides*. Four species (C. *flexuosus, Cymbopogon citratus, Coriandrum sativum* and *Lippia alba*) showed better antifungal activity. Citral is the main component of essential oils of most species of *Cymbopogon* species, and many authors attribute to this compound the control of plant pathogenic fungi. Valencia et al. (2011) found antifungal effect of essential oil of *C. citratus* on *C. gloeosporioides*. Alzate (2009) demonstrated that citral completely inhibited mycelial growth and sporulation of *C. acutatum* outperforming Mancozeb fungicide. According to Bakkali et al. (2008), as these oxygenated monoterpenes are hydrophobic they will probably want to move towards the aqueous phase of membrane structures. Accumulation of essential oil constituents in the lipid bilayer of the cytoplasmic membrane makes it permeable, thus promoting dissipation of the proton motive force. It also reduces ATP pool, internal pH and electric potential, causing loss of ions such as potassium and phosphate. Thus, damage leads to impairment of membrane functions.

According to Rasooli et al. (2006), when in direct contact with microorganisms, these substances cause permeability of cell membranes and leakage of their contents. Furthermore, terpene alcohols were identified in EOC composition, such as geraniol and linalool (Table 1).

Few studies have reported antimicrobial activity of species of *Vernonia* spp. Essential oil of *V. polyanthes* presented lower antifungal activity than *C. flexuosus* oil (Figure 1B and D). These results demonstrate that microorganisms differ in their resistance to certain essential

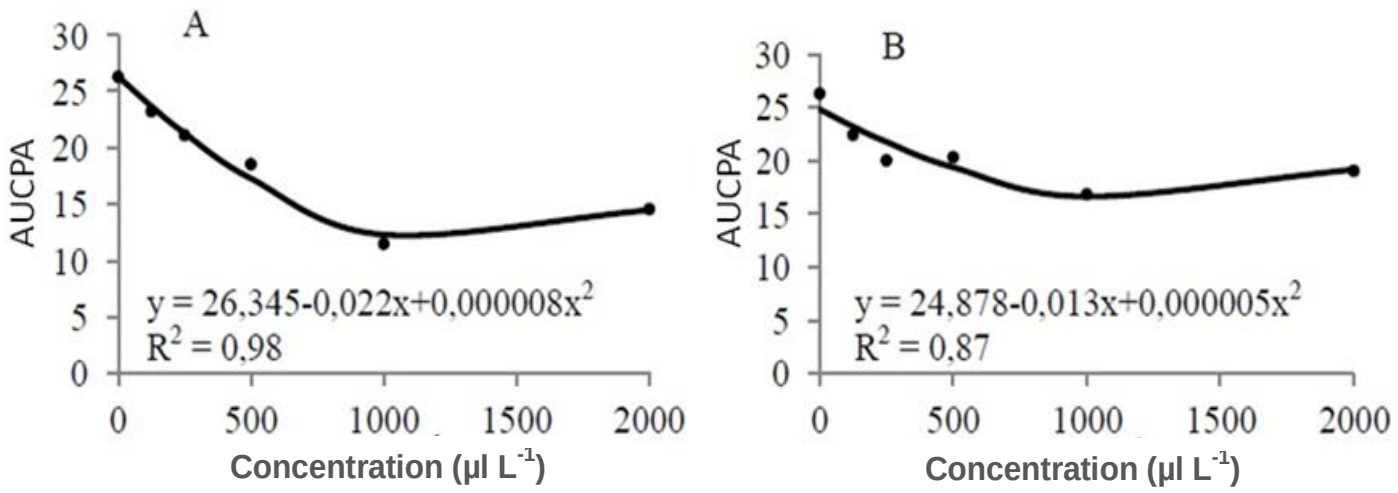

Figure 2. Area under the curve of progress of anthracnose (AUCPA) 35 days after inoculation of *C. lindemuthianum* in bean plants cultivar Pérola, treated with different concentrations (0, 125, 250, 500, 1000 and 2000 µL L^{-1}) of essential oils of *C. flexuosus* (A) and *V. polyanthes* (B). Data were transformed to √x + 0.5.

oils, showing specific reaction according to the chemical constitution of each oil.

Maia et al. (2010) evaluated essential oils of *Vernonia braziliana* and *Vernonia remotiflora* in Gram-negative and Gram-positive bacteria. The antimicrobial activity of these oils is related to the high amount of sesquiterpenes such as those found in this study: germacrene-D, bicyclo-germacrene, β-caryophyllene, and α-humulene (Table 1).

Montanari et al. (2011) attributed antimicrobial activity to sesquiterpene due to inhibition of breathing capacity and increased cell membrane permeability, as well as rupture of membrane integrity which results in leakage of K + ions and consequent loss of chemiosmosis control. Oils of *Lantana camara* and *Aloysia virgata*, rich in germacrene-D, showed moderate antibacterial activity against Gram-positive bacteria (*B. cereus* and *S. aureus*) and oil of *A. virgata* was active against Gram-negative bacteria (Costa et al., 2009).

Essential oil of *C. citratus* reduced the severity of anthracnose in passion fruit caused by *C. gloeosporioides*, with no significant difference in relation to Procloraz fungicide (Anaruna et al., 2010) or *Hibiscus rosa-sinensis* (Valencia et al., 2011).

Some authors attribute the partial control of other anthracnoses to citral. However, it is necessary to conduct experiments testing the isolated effect of neral and geranial. Garcia et al. (2008) reported that citral 1% was responsible for 70% control of anthracnose in papaya and 60% of anthracnose in banana. Essential oils rich in terpenes cause damage to lipids and proteins and break down cell walls and membranes, which results in cell lysis. In eukaryotic cells, essential oils destabilize the mitochondrial membrane and cause damage to plasma membrane proteins (Bakkali et al., 2008).

Direct effectiveness of phosphites against fungi is mainly related to phosphite ion, which seems to have a direct effect on the pathogen (Pereira et al., 2012). Furthermore, phosphites can also reduce sporulation of microorganisms, thereby causing reduction of pathogen inoculum potential (Silva et al., 2012).

The results of scanning electron microscopy in this study corroborate the results found in conidia germination *in vitro*, which had inhibition of germination with EOC, PP and EOV. Other authors have used SEM to demonstrate antifungal activity of essential oils (Pereira et al., 2011) treated leaves of coffee with *Cinnamomum zeylanicum* and *C. citratus* oils in control of *Cercospora coffeicola*. Through SEM observations, the authors found reduction in germination and mycelial size, with plasmolysis occurring in some conidia.

Induction of resistance in essential oils (EOs) may be related to their chemical constitution. It is known that EOs have various organic substances capable of inducing POX activity, such as terpene hydrocarbons, terpene alcohols, and simple alcohols, aldehydes, ketones, phenols, esters, ethers, oxides, peroxides, furans, organic acids, lactones, coumarins, and sulfur-containing compounds (Dewick, 2002). Thus, by chromatography data (Table 1), the presence of some of these substances which probably contributed to the increased levels of POX in bean tissues were confirmed. Pereira et al. (2012) found increased POX enzyme in coffee plants sprayed with essential oil of citronella, which resulted in decreased Cercospora leaf spot.

According to Daniel and Guest (2006), after treatment with phosphite there is accumulation of phenolic compounds in cells, formation of cytoplasmic aggregates and phenols around the infected cells and rapid increase in production of reactive oxygen species, followed by hypersensitivity reactions.

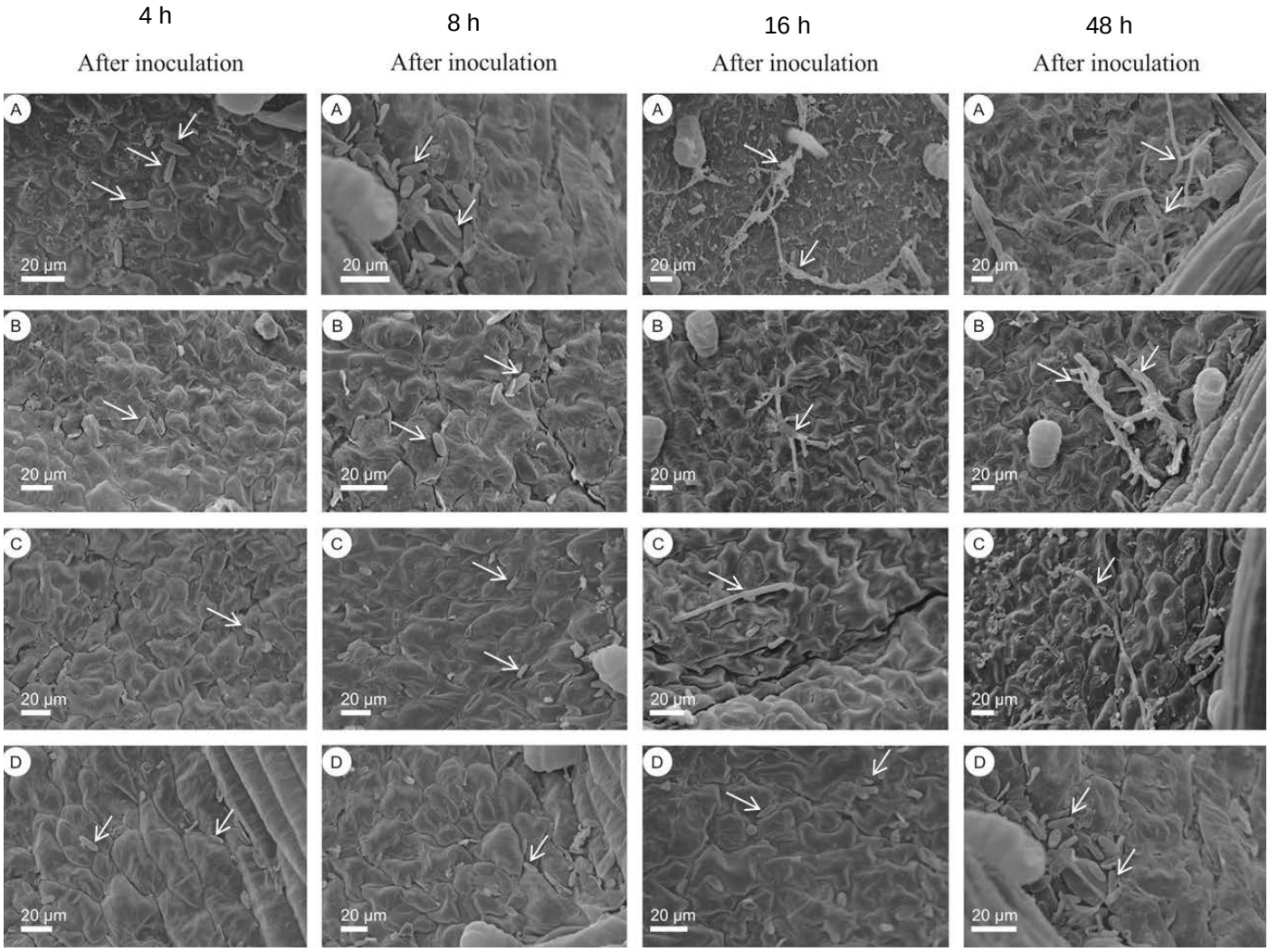

Figure 3. Scanning electron microscopy of bean leaves inoculated with *C. lindemuthianum* in different times after inoculation. Leaves were sprayed with distilled water (A), essential oil of *V. polyanthes* 1000 µl L^{-1} (B), *C. flexuosus* 1000 µl L^{-1} (C), and potassium phosphite 5 ml L^{-1} (D).

The positive relationship between POX activity of plant resistance to disease has been reported in several studies. Thus, increased POX activity could explain the lower severity of bean anthracnose found in plants treated with PP and EOC in severity experiments. Martins et al. (2013), also studying Pérola cultivar found increased activity of POX in plants treated with rhizobacteria and inoculated with *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*. The authors also found decreased severity of disease at levels 42 to 76%.

Campos et al. (2004) reported that POX activity was significantly higher in bean plants treated with salicylic acid before and after inoculation of *C. lindemuthiaum*, with positive correlation between increased POX activity and anthracnose resistance. This resistance is related to POX ability to produce free radicals toxic to the pathogen in oxidative burst and to participate in lignin synthesis for strengthening cell wall. In addition, POX produces signal molecules such as H_2O_2, which can lead to expression of genes related to other resistance mechanisms (Hsu and Kao, 2003).

There was a significant difference regarding the activity of enzyme phenylalanine ammonia-lyase (PAL). Inoculated plants sprayed with PP had greater activity of PAL compared to plants sprayed with EOC and water at 8 and 10 days after spraying (Table 5). At day 13, plants sprayed with PP produced less PAL and did not differ from oil. This was possibly due to increase in PAL in early periods post inoculation (at 8 and 10 days after spraying), which caused a metabolic cost to the plant on the last evaluation day. This same effect occurred with plants treated with water, that is, PAL activity decreased considerably on the last day after inoculation (day 13). However, enzyme activity was lower than the activity of

elicitors PP and EOC, which resulted in increased severity of anthracnose in plants treated with water. Similar to PP treatment, inoculated plants sprayed with EOC showed higher PAL activity in all periods. This increase can mean that the entire phenylpropanoid pathway was altered, that is, mechanisms such as synthesis of lignin, phenolic compounds, quinones and others may have been potentialized by the products used in the experiment.

Non-inoculated plants showed no statistical difference between products at 8 and 13 days after spraying. Only at day 10, plants sprayed with PP had higher PAL activity. Thus, it is evident that PAL peak occurred at day 10. The highest PAL activity in plants treated with PP and essential oils has already been reported by other authors. Sellamuthu et al. (2013) found PAL to increase in avocado fruits with application of essential oil of thyme. Martins et al. (2013) reported increase in PAL levels in the second phenological stage of bean plants Pérola cultivar treated with growth promoting rhizobacteria. The authors also reported decreased severity of wilt of *C. flaccumfaciens* pv. *flaccumfaciens* in common bean by 76%, and increased dry weight of shoots and roots of treated plants. Campos et al. (2003) reported that bean plants Pérola cultivar treated with salicylic acid had higher PAL activity than plants treated with water, and plants challenged with the pathogen *C. lindemuthiahum* showed higher PAL activity than non-inoculated plants.

PAL catalyzes the deamination reaction of L-phenylalanine to trans-Cinnamic acid. This process is the first step in the phenylpropanoid pathway which is highly important for the production of plant defense compounds against pathogens (Mandal et al., 2009).

Thus, essential oil of *C. flexuosus* and potassium phosphite could be an alternative for the management of bean anthracnose, because in addition to significant induction of defense enzymes such as POX and PAL, these products also presented antifungal proprieties. However, more studies need to be performed to assess the efficiency of these products in anthracnose control in field conditions, doses to be used and toxicology to man and the environment, as well as the adequacy of essential oil production and oil formulation.

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Conflict of interests

The author(s) have not declared any conflict of interests.

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In vitro anti-*Salmonella* activity of extracts from selected Kenyan medicinal plants

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The aim of this study was to determine *in vitro* anti-*Salmonella* activity of extracts of five selected Kenyan medicinal plants against *Salmonella* ser. Typhi and *Salmonella* ser. Typhimurium. The extracts from *Tithonia diversifolia, Warburgia ugandesis, Croton megalocarpus*, *Carissa edulis* and *Launae cornuta* plants traditionally used in treatment of typhoid fever were screened for anti-*Salmonella* activity using disc diffusion and microdilution techniques. The results from the present study have shown that out of thirty six extracts investigated, only nine extracts from *T. diversifolia* and *W. ugendensis* showed activity against *Salmonella* ser. Typhi and *Salmonella* ser. Typhimurium at 1000 mg/ml. The inhibition zone of ethyl acetate, hexane and methanol extracts of *T. diversifolia* leaves, ethyl acetate and hexane extracts of *T. diversifolia* flowers, ethyl acetate and hexane extracts of *W. ugandensis* stem barks, ethyl acetate and hexane extract of *W. ugandensis* roots ranged from 8 to 18.5 ± 0 mm. These results were comparable with those of ciprofloxacin (19.67 to 26 mm) and chloramphenicol (6.67 to 24.33 mm). The minimum inhibitory concentration (MIC) of the active extracts were in the range of 0.031 to 15.63 mg/ml which compared very well with ciprofloxacin (0.015 to 0.02) and chloramphenicol (0.022 to 0.03 mg/ml). Extracts with anti-*Salmonella* activity can be used to source antibiotic substances useful in the treatment of typhoid fever. The study provides the scientific basis for the traditional application against typhoid fever.

Key words: Anti-*Salmonella* activity, medicinal plant extracts, minimum inhibitory concentration, disc diffusion technique, microdilution technique, *Salmonella* strains, typhoid.

INTRODUCTION

Salmonella serotype Typhimurium (*S.* ser. Typhimurium), is a Gram-negative bacterial pathogen that infects humans and animals, causing significant morbidity and mortality worldwide (Fink and Cookson, 2007). It is an obligate intracellular bacterial pathogen that causes gastroenteritis in millions of people worldwide each year (Grassl et al., 2008). For instance, the Centre for Disease Control (CDC) estimates that there are nearly 1.4 million food-borne *Salmonella* infections annually in the USA (Mead et al., 1999). Various strategies have been

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employed in the treatment and management of *Salmonella* infection. Fluoroquinolones and tetracyclines are most commonly used to treat *Salmonella* infections. However, *Salmonella* strains resistant to these antibiotics have been reported in Korea and other countries (Choi et al., 2005; Stevenson et al., 2007). One major concern to public health has been the global dissemination of *S.* typhimurium Definitive Type 104, which is resistant to cotrimoxazole, nalidixic acid and ampicilin (Perron et al., 2008; Kariuki et al., 2010). The rise in antibiotic-resistant strains has led to increased interest in use of plant materials to develop new effective drugs. According to World Health Organization (WHO) more than 80% of world's population relies on traditional medicine for their primary healthcare, majority of who use plant active principles (Gupta et al., 2005). A wide variety of plants are used in Africa for treatment of fever, dysentry, cholera, diarrhoea and other infections typical of the tropical countries (Ayogu and Amadi, 2009; Ajayi and Akintola, 2010). For instance, traditional practitioners in Nigeria use herbal preparations to treat microbial infections such as typhoid and paratyphoid infections (Iroha et al., 2010).

Plants used in this study have traditionally been associated with disease curative and preventive practices in many countries for a long time. Garcia and Delgado (2006) have reported that *Tithornia diversifolia* has promising medicinal value. Skin products formulated from *T. diversifolia* extracts have been shown to have antimicrobial properties (Kareru et al., 2010).

In Ethiopia *Warburgia ugandensis* extracts are used to treat malaria, tuberculosis, bronchitis, pneumonia, hepatitis, tapeworm, gonorrhea and asthma (Wube et al., 2010; Were et al., 2010; Opiyo et al., 2011). The decoction from *Croton megalocarpus* bark is used as a remedy for worms and whooping cough. Grounded roots are used for syphilis, anthrax, and snakebites treatment (Kabir et al., 2005). Different communities in Africa use parts of *Carissa edulis* to alleviate pain, treat venereal diseases, glandular inflammation, induce abortion and restore virility (Githiori et al., 2004). In Kenya and Tanzania decoction from *Launae cornuta* roots is used as a remedy for cough, typhus fever and measles (Schippers, 2004).

This study investigated the anti-*Salmonella* activities of *T. diversifolia, W. ugandensis*, *C*. *megalocarpus*, *C. edulis* and *Launae cornuta*. Clinical isolates of *S.*ser.Typhi (ATCC 13347), *S.*ser.Typhi (ATCC 43579), *S.enterica* (ATCC 2162) and *S.* ser. Typhimurium (ATCC 1408) were used in the study.

MATERIALS AND METHODS

Salmonella strains

Clinical samples of *S.*ser.Typhi (ATCC 13347), *S.*ser.Typhi (ATCC 43579), *S.enterica* (ATCC 2162) and *S.* ser. Typhimurium (ATCC 1408) were provided by the Centre of Microbiology Research, Kenya Medical Research Institute (CMR-KEMRI) for this study.

Plant

The five plants selected for this study were collected in Nyamira County as indicated in Table 1. The plants were authenticated at Jomo Kenyatta University of Agriculture and Technology, Botany Department.

Experimental design

The nine plant parts obtained from 5 selected medicinal plants indicated in Table 1 were extracted using four solvent systems namely; hexane, ethyl acetate, methanol and water. The extracts obtained were subjected to standard phytochemical analyses as described by Jigna et al. (2006). In addition, the extracts were screened for anti-*Salmonella* activity against four clinical isolates. Samples in triplicate were subjected to disc diffusion and microdilition in duplicates to confirm anti-*Salmonella* activity.

Preparation of plant materials for extraction

The plant materials were washed under running tap water and left to drain off. The plant parts were chopped into small pieces. The dried pieces were ground into powder and their weights recorded.

Extraction techniques

The nine plant materials were extracted using selected solvents. Each plant material was extracted sequentially using hexane, ethyl acetate and methanol in the order of increasing polarity. Single extraction was carried out using water for each of the nine plant materials.

Preparation of hexane extract

Approximately 500 g of each plant powder was soaked separately in 1500 ml of hexane. The contents were kept for 3 days away from direct sunlight, undisturbed, then filtered through sterile filter paper. The filtrate was concentrated at 38.5 to 42°C.

Preparation of ethyl acetate extract

The hexane residues were re-soaked in 1500 ml of ethyl acetate. The contents were kept for 5 days away from direct sunlight, undisturbed and afterward filtered. The filtrate was concentrated at 38.5 to 42°C.

Preparation of methanol extract

The ethyl acetate residues were re-soaked in 1500 ml of methanol and kept for 36 h away from direct sunlight undisturbed. After filtration, the filtrate was concentrated at 65°C. The extracts were stored at 4°C until used.

Preparation of aqueous extract

Five hundred grams (500 g) of each of the powdered plant materials was weighed and soaked separately in 1500 ml of distilled water. The contents were warmed in a water bath for 2 h at

Table 1. Profile of the five medicinal plants.

Botanical name	Family name	Part of the plant used
Tithornia diversifolia	Solanaceae	Flowers and leaves
Warburgia ugandesis	Conellaceae	Roots and stem barks
Croton megalocarpus	Euphorbiaceae	Barks
Carissa edulis	Apocynaceae	Roots and barks
Launae cornuta	Asteraceae	Roots and leaves

60°C, then left to stand at room temperature for 10 h, undisturbed. They were subsequently sterile filtered and filtrate freeze dried to powder. The powder were weighed and stored until used.

Determination of phytochemical constituents

The freshly prepared extracts were subjected to standard phytochemical analyses for tannins, alkaloids, terpenoids, flavanoids, glycosides, steroids and saponin as described by Jigna et al. (2006).

Controls

Water and dimethyl sulfoxide (DMSO) were used as negative controls. Ciprofloxacin and chloramphenicol (Transchem pharmaceutical Ltd, Kenya) were used as positive controls.

Disc diffusion assay

Circular paper discs (6mm diameter) were placed on Muller Hinton media inoculated with *Salmonella* strains. Sterile paper discs were dampened with 10 µl of plant extracts at 1000 mg/ml. The loaded disc was placed on the surface of the medium, the compound was allowed to diffuse for 5 min and plates were incubated for 24 h at 37°C. Discs containing ciprofloxacin and chloramphenicol were used as positive controls. Discs loaded with DMSO and water served as negative controls. The assays were performed in triplicate. Anti-*Salmonella* activity was evaluated by measuring diameter of the inhibition zone.

Determination of minimum inhibitory concentration (MIC) values

The MIC values were determined using microdilution assay as described by Eloff (1998). Ciprofloxacin and chloramphenicol were used as positive controls and DMSO was used as negative control. Plant extracts were tested against *Salmonella* strains with varying concentration ranging from 62.5 to 0.0305 mg/ml. Briefly, 100 µl of sterile distilled water was added to each well of 96-well microtitre plates (SIGMA Aldrich, German) followed by the addition of 100 µl of 62.5 mg/ml and thereafter serially diluted plant extracts. Then 100 µl of *Salmonella* strains were added to each micro well to give a final volume of 200. The prepared plates were sealed to avoid drying and incubated overnight at 37°C. After overnight incubation, 50 µl of 5 mg/ml 2, 3, 5 triphenyltetrazolium chloride (SIGMA Aldrich, German) was added to the wells and incubated overnight. The pink colour was indicative of bacterial growth while lack of color was linked to growth inhibition. The MIC was defined as the lowest concentration of plant extract that completely suppress the growth of *Salmonella* strains.

Statistical analysis

Anti-*Salmonella* activity was determined from means of triplicates in zones of inhibition and duplicates in MICs. Collected data was analysed statistically using one way ANOVA (SAS, Version 9.0). Difference in values at $P < 0.0001$ were considered statistically significant.

RESULTS

Out of 36 plant extracts screened using disc diffusion assay, only nine extracts inhibited the growth of clinical *Salmonella* organisms at 1000 mg/ml. Extracts of hexane (flowers), ethyl acetate (leaves) and methanol (leaves) extracts from *T. diversifolia* were active against *S*.ser.Typhi ATCC 13347, *S*.ser.Typhi ATCC 43579, *S.enterica* ATCC 2162 and *S*.ser.Typhimurium ATCC 1408. Extracts of hexane (leaves) and ethyl acetate (flowers) from *T. diversifolia* inhibited growth of *S.*ser.Typhi ATCC13347. As was observed with ciprofloxacin and chloramphenicol controls, extracts of hexane and ethyl acetate (roots and stem bark) from *W. ugandensis* inhibited growth of all the tested *Salmonella* organisms. Extracts of methanol (leaves) from *T. diversifolia* were also observed to inhibit all the clinical isolates at 8 to 12 mm. The zones of inhibition for the active extracts are shown in Table 2.

The MIC values of the nine plant extracts was evaluated and shown to range from 0.031 to 15.63 mg/ml. The MICs of hexane extracts from *T. diversifolia* leaves and flowers ranged from 0.24 to 1.95 mg/ml and 0.12 to 3.91 mg/ml, respectively. The MICs of hexane extracts from *W. ugandensis* roots and stem bark ranged from 0.031 to 3.91 mg/ml and 0.031 to 0.488 mg/ml, respectively. Table 3 shows MICs of the nine plant extracts and controls. It is evident from these results that *W. ugandensis* extracts were the most active against all the *Salmonella* strains tested.

Table 4 illustrates anti-*Salmonella* activity values obtained from disc diffusion and microdilution methods. The nine plant extracts showed different value of anti-*Salmonella* activity against test strains. Ethyl acetate extract of *W. ugandensis* stem bark gave the lowest MIC value of 0.031 mg/ml against *S*.ser.Typhi ATCC 13347, *S*.ser.Typhi ATCC 43579, and *S*.ser.Typhimurium ATCC 1408 with zones of inhibition of 6, 7 and 7.33 mm,

Table 2. Zones of inhibition of clinical *Salmonella* strains by hexane, ethyl acetate and methanol extracts of selected medicinal plants

Plant extracts	Mean diameter of inhibition zones (mm)			
	***S*.ser.Typhi ATCC 13347**	***S*.ser.Typhi ATCC 43579**	***S.enterica* ATCC 2162**	***S.* ser. Typhimurium ATCC 1408**
TDLE	10 ± 0^{hij}	10 ± 0.58^{hij}	7.33 ± 0.58^{kl}	7.33 ± 2.31^{kl}
TDFH	15.67 ± 2.08^{g}	15.75 ± 1.15^{g}	7.33 ± 1.15^{kl}	6 ± 0^{l}
TDLM	11 ± 1^{h}	11.5±58h	$7.33\pm4.0.58^{kl}$	11.67 ± 0.58^{h}
TDLH	17.67 ± 2.08^{f}	17 ± 0^{f}	6 ± 0^{l}	6 ± 0^{l}
TDFE	18 ± 2^{f}	18.5 ± 0^{f}	6 ± 0^{l}	6.67 ± 0.58^{kl}
WURE	8.67 ± 0.58^{ijk}	6 ± 0^{l}	6.67 ± 0.58^{kl}	6.67 ± 1.15^{kl}
WURH	14 ± 1^{g}	8.33 ± 0.58^{jkl}	6.67 ± 1.15^{kl}	10.67 ± 4.62^{hi}
WUSBE	6 ± 0^{l}	7 ± 0^{kl}	6.33 ± 0.58^{kl}	6 ± 0^{l}
WUSBH	11 ± 3.21^{h}	7.33 ± 2.31^{kl}	6.33 ± 0.58^{kl}	7.33 ± 0.58^{kl}
DMSO(-)	6 ± 0^{l}	6 ± 0^{l}	6 ± 0^{l}	6 ± 0^{l}
CHLO(+)	23.33 ± 0.58^{de}	24 ± 1.73^{cde}	24.33 ± 0.58^{cde}	8.67 ± 0.58^{jk}
CIPRO(+)	26 ± 2^{abc}	23.33 ± 2.52^{a}	26 ± 0^{abc}	19.67 ± 1.53^{f}

IZ = Inhibition zone (in mm) includes the diameter of the disc, TDLE = *Tithonia diversifolia* leaf extract of ethyl acetate, TDLH = *Tithonia diversifolia* leaf extract of hexane, TDLM = *Tithonia diversifolia* leaf extract of methanol, TDFH = *Tithonia diversifolia* flower extract of hexane, TDFE = *Tithonia diversifolia* flower extract of ethyl acetate, WURE = *Warburgia ugandensis* root extract of ethyl acetate, WURH = *Warburgia ugandensis* root extract of hexane, WUSBE = *Warburgia ugandensis* stem bark extract of ethyl acetate, WUSBH = *Warburgia ugandensis* stem bark extract of hexane, DMSO(-)=Dimethyl sulphur dioxide (Negative control), CIPRO(+) = Ciprofloxacin (Positive control), CHLO(+) = Chloramphenicol (Positive control), Values are means of triplicate readings (Means ± SD). Means followed by different superscript letters in the table above are significantly different at $P < 0.0001$.

respectively (Table 4). The extract showed MIC of 0.061 mg/ml against *S.enterica* ATCC 2162 with inhibition zone of 6 mm. The *T. diversifolia* extracts had anti-*Salmonella* activity against all the tested clinical isolates. Methanol extract of the leaves showed activity with MIC values of 0.031, 0.24, 0.448 and 0.98 mg/ml against *S*.ser.Typhi ATCC 43579, *S*. ser. Typhi ATCC 13347, *S*. ser. Typhimurium ATCC 1408 and *S. enterica* ATCC 2162 with inhibition zones of 11.5, 11, 11.67 and 7.33, respectively.

The nine active plant extracts were screened further to study the presence of medicinally active phytochemicals in leaves, stem barks, roots and flowers. Phytochemical analysis revealed presence of alkaloids, saponin, tannins, flavanoids, steroids, terpenoids and glycosides (Table 5). Steroids were detected in all the extracts. Flavonoids and tannins were absent in hexane extracts of *T*. *diversifolia* flower and *W*. *ugandensis* stem bark. Terpenoids were found in extracts of hexane and ethyl acetate from *T*. *diversifolia* flower, *W*. *ugandensis* root and *W*. *ugandensis* stem bark. Extracts of hexane (*T*. *diversifolia* flower) and (*W*. *ugandensis* stem bark) lacked alkaloids. Glycosides were detected in extracts of hexane and ethyl acetate (*T*. *diversifolia* leaf) and ethyl acetate (*W*. *ugandensis* stem bark). Saponins were detected only in extracts of methanol from *T*. *diversifolia* leaf (Table 5).

DISCUSION

In the present study, the extracts from 5 medicinal plants were screened for activity against clinical *Salmonella* strains. Of the extracts tested, both ethyl acetate and hexane extracts of *T. diversifolia* and *W. ugandensis* exhibited activity against all four *Salmonella* strains tested in this study. Methanol extracts of *T. diversifolia* leaf also inhibited all the clinical isolates tested.

T. diversifolia plant extracts exhibited different zones of inhibition against the isolates. The ethyl acetate flower and hexane leaf extracts of *T*. *diversifolia* gave zones of inhibition at 18.5 ± 5 mm and 17.67 ± 2 mm, respectively (Table 2). This compared well with ciprofloxacin which gave zone of inhibition of 19.67 mm and therefore no significant difference in activity ($p < 0.0001$). Methanol extract of *T*. *diversifolia* leaf also showed anti-*Salmonella* activity against test isolates. The observed anti salmonella activity of *T. diversifolia* extracts agrees with the finding of Ogunfolakan et al. (2010), on broad spectrum antimicrobial activity. Kareru et al. (2010) has reported that soap made from leaf extract of *T. diversifolia* was effective against *E. coli*.

The phytochemicals and secondary metabolites from plants possess antimicrobial activity (Srikumar et al., 2007). Phytochemical analysis demonstrated the presence of alkaloids, tannin, flavonoids, terpenoids steroids and glycosides in the active extracts. Ethyl acetate flower extracts exhibited the highest anti-*Salmonella* activity. This is due to the difference in the type and concentrations of secondary metabolites in different plant parts (Srikumar et al., 2007) and may be contributing to the observed differences in anti-*Salmonela*

Table 3. Minimum Inhibitory Concentration (mg/ml) of Hexane, ethyl acetate and methanol extracts

Plant extracts	*Salmonella* organisms			
	***S.*ser.Typhi ATCC 13347**	***S.*ser.Typhi ATCC 43579**	***S.enterica* ATCC 2162**	***S.* ser. Typhimurium ATCC 1408**
	mg/ml	**mg/ml**	**mg/ml**	**mg/ml**
TDLE	0.24^{f}	0.061^{h}	0.031^{j}	0.98^{d}
TDFH	0.98^{d}	0.12^{g}	3.91^{b}	3.91^{b}
TDLM	0.24^{f}	0.031^{j}	0.98^{d}	0.488^{e}
TDLH	0.24^{f}	0.24^{f}	1.95^{c}	0.488^{e}
TDFE	0.98^{d}	15.63^{a}	0.12^{g}	3.91^{b}
WURE	0.24^{f}	0.031^{j}	0.061^{h}	0.12^{g}
WURH	0.031^{j}	0.031^{j}	3.91^{b}	0.031^{j}
WUSBE	0.031^{j}	0.031^{j}	0.061^{h}	0.031^{j}
WUSBH	0.031^{j}	0.031^{j}	0.488^{e}	0.0467^{i}
DMSO(-)	ND	ND	ND	ND
CHLO(+)	0.022^{klm}	0.029^{jk}	0.024^{ijk}	0.030^{j}
CIPRO(+)	0.02^{lm}	0.015^{m}	0.018^{lm}	0.025^{ijk}

ND =Not defined, TDLE=*Tithonia diversifolia* leaf extract of ethyl acetate,TDFH= *Tithonia diversifolia* flower extract of hexane, TDLM= *Tithonia diversifolia* leaf extract of methanol, TDLH= *Tithonia diversifolia* leaf extract of hexane, TDFE= *Tithonia diversifolia* flower extract of ethyl acetate, WURE=*Warburgia ugandensis* root extract of ethyl acetate, WURH= *Warburgia ugandensis* root extract of hexane, WUSBE= *Warburgia ugandensis* stem bark extract of ethyl acetate, WUSBH= *Warburgia ugandensis* stem bark extract of hexane, DMSO(-VE)=Dimethyl sulphur dioxide (Negative control), CIPRO(+VE)=Ciprofloxacin(Positive control), CHLO(+VE)=Chloramphenicol(Positive control), Values are means of duplicate readings. Means followed by different superscript letters in the table above are significantly different at P<0.0001.

activity. The results of this study show that extract with high content of steroids contribute to significant inhibition of *Salmonella* growth. This finding agrees with Ashok and Vijayalakshmi (2013) who have demonstrated that sterols from *Vitis vinifera* seed exhibited antibacterial activity. The sterols could be interacting with the bacterial cell wall and membrane ultimately leading to pore formation and disrupting bacterial membrane integrity (Devjani and Barkha, 2011). Odeyemi et al. (2014) has also reported that *T. diversifolia* leaf, flower and roots extracts have antibacterial activity due to the presence of metabolic toxins such as flavonoids, steroids and alkaloids.

Hexane extracts of *W. ugandensis* roots and stem barks showed inhibition zones of 14 and 11 mm, respectively against *S*.ser.Typhi ATCC 13347. Hexane extract of *W. ugandensis* roots and chloramphenicol showed inhibition zones of 10.67 and 8.67 mm against *S*. ser. Typhimurium ATCC 1408, respectively (Table 2). This was significantly lower than that of ciprofloxacin (19.67 mm). The observed anti-*Salmonella* activity of *W. ugandensis* is however supported by Yibeltal et al. (2013) who demonstrated activity of crude and semi-purified fractions of *W. ugandensis* against *Shigella boydii* and *Staphylococcous aureus*. Studies carried out by Olila et al. (2001) on aqueous extracts of *W. ugandensis* stem bark showed activity against both *Escherischia coli* and *S. aureus* in agar well assays but not in disc diffusion assay. The anti-*Salmonella* activity of *W. ugandensis* observed in our present study could be attributed to several secondary metabolites, among them steroids.

The extracts of three plants namely *Croton megalocarpus, Croton edulis* and *Lactoria cornuta* had no anti-*Salmonella* activity for the extracts (Table 2). Their anti-*Salmonella* activity values were not significantly different with those of negative controls ($p < 0.0001$). The lack of anti-*Salmonella* activity in these plants may not necessarily imply the same *in vivo* since compounds may either act as pro-drug which must undergo metabolic changes to achieve the required activity. Besides, the presence of bioactive compounds depends on many factors such as the season, age, intra-species variation, part of the plant collected, soil and climate (Gessier et al., 1995).

The MIC values of the nine active plant extracts was determined. These extracts were selected because of their appreciable anti-*Salmonella* performance determined by disc diffusion. The active extracts against *Salmonella* strains were *W. ugandensis* bark, *W. ugandensis* root, *T. diversifolia* leaf and *T. diversifolia* flower. The ethyl acetate extract of *W. ugandensis* stem bark showed anti-*Salmonella* activity among the extracts tested. The extract had MIC value of 0.031 mgl/ml

Table 4. Mean anti-*Salmonella* activity values obtained by disc diffusion and microdilution technique for the active plant extracts against *Salmonella* strains.

Plant extracts		*Salmonella* organisms			
		***S.*ser.Typhi ATCC 13347**	***S.*ser.Typhi ATCC 43579**	***S.enterica* ATCC 2162**	***S.* ser. Typhimurium ATCC 1408**
TDLE	MIC (mg/ml)	0.24	0.061	0.031	0.98
	IZ (mm)	10±0	10±0.58	7.33±0.58	7.33±2.31
TDFH	MIC (mg/ml)	0.98	0.12	3.91	3.91
	IZ (mm)	15.67±2.08	15.75±1.15	7.33±1.15	6±0
TDLM	MIC (mg/ml)	0.24	0.031	0.98	0.488
	IZ (mm)	11±1	11.5±0.58	7.33±1.15	11.67±0.58
TDLH	MIC (mg/ml)	0.24	0.24	1.95	0.488
	IZ (mm)	17.67±2.08	17±0	6±0	6.67±0.58
TDFE	MIC (mg/ml)	0.98	15.63	0.12	3.91
	IZ (mm)	18±2	18.5±0	6±0	6.67±0.58
WURE	MIC (mg/ml)	0.24	0.031	0.061	0.12
	IZ (mm)	8.67±0.58	6±0	6.67±0.58	6.67±1.15
WURH	MIC (mg/ml)	0.031	0.031	3.91	0.031
	IZ (mm)	14±1	8.33±0.58	6.67±1.15	10.67±4.62
WUSBE	MIC (mg/ml)	0.031	0.031	0.061	0.031
	IZ (mm)	6±0	7±0	6.33±0.58	6±0
WUSBH	MIC (mg/ml)	0.031	0.031	0.488	0.0467
	IZ (mm)	11±3.2	7.33±2.31	6.33±0.58	7.33±0.58
DMSO(-)	MIC (mg/ml)	ND	ND	ND	ND
	IZ (mm)	6±0	6±0	6±0	6±0
CHLO(+)	MIC (mg/ml)	0.022	0.029	0.024	0.030
	IZ (mm)	23.33±0.58	24±1.73	24.33±0.58	8.67±1.53
CIPRO(+)	MIC (mg/ml)	0.02	0.015	0.018	0.025
	IZ (mm)	26±2	23.33±2.52	26±0	19.67±1.53

MIC= minimum inhibitory concentration (mg/ml), IZ=Inhibition zones (mm), TDLE=*Tithonia diversifolia* leaf extract of ethyl acetate, TDFH= *Tithonia diversifolia* flower extract of hexane, TDLM= *Tithonia diversifolia* leaf extract of methanol, TDLH= *Tithonia diversifolia* leaf extract of hexane, TDFE= *Tithonia diversifolia* flower extract of ethyl acetate, WURE=*Warburgia ugandensis* root extract of ethyl acetate, WURH= *Warburgia ugandensis* root extract of hexane, WUSBE= *Warburgia ugandensis* stem bark extract of ethyl acetate, WUSBH= *Warburgia ugandensis* stem bark extract of hexane, and CMBM= *Croton megalocarpu*s bark extract of methanol, DMSO (-VE)=Dimethyl sulphur dioxide (Negative control), CIPRO(+VE)=Ciprofloxacin(Positive control), CHLO(+VE)=Chloramphenicol(Positive control).

against *S*.ser.Typhi ATCC 13347, *S*.ser.Typhi ATCC 43579, and *S*.ser.Typhimurium ATCC 1408. It showed MIC value of 0.061 mg/ml against *S. enterica* ATCC 2162. Hexane extract of *W. ugandensis* root showed MIC value of 0.031 mg/ml against *Salmonella* strains tested except *S. enterica* ATCC 2162 which was inhibited at 3.91 mg/ml. The extracts of hexane (stem bark) and ethyl acetate (root) from *W. ugandensis* showed anti-Salmonella

Table 5. Phytochemical constituents of the active plant extracts.

Plant extracts	Alkaloids	Saponin	Tannins	Flavanoids	Steroids	Terpenoids	Glycosides
TDLE	+	-	+	+	+++	-	+
TDFH	-	-	-	-	+++	+	-
TDLM	+	++	++	+	+++	-	-
TDLH	+	-	++	+	+++	-	+
TDFE	+	-	+	+	+++	++	-
WURE	+	-	+	+	++	++	-
WURH	-	-	+	+	++	++	-
WUSBE	+	-	+	+	++	+	++
WUSBH	-	-	-	-	++	++	-

- = absent; + = present; ++ = Moderate concentration; +++ = High concentration. TDLE=*Tithonia diversifolia* leaf extract of ethyl acetate, TDFH= *Tithonia diversifolia* flower extract of hexane, TDLM= *Tithonia diversifolia* leaf extract of methanol, TDLH= *Tithonia diversifolia* leaf extract of hexane, TDFE= *Tithonia diversifolia* flower extract of ethyl acetate; WURE=*Warburgia ugandensis* root extract of ethyl acetate, WURH= *Warburgia ugandensis* root extract of hexane, WUSBE= *Warburgia ugandensis* stem bark extract of ethyl acetate, WUSBH= *Warburgia ugandensis* stem bark extract of hexane, and CMBM= *Croton megalocarpu*s bark extract of methanol.

salmonella activity against all strains tested (Table 3). Anti-*Salmonella* activity of *W. ugandensis* extracts compared well with ciprofloxacin and chloramphenicol. In a study carried out by Yibeltal et al. (2013), on antimicrobial activity of crude extracts of *W. ugandensis* against *E. coli* and *P. aeruginosa* demonstrated MIC values of 1.75 mg/ml. These results compared well with those of our study, which were in the range of 0.031 to 3.91 mg/ml (Table 3).

The methanol extract of *T. diversifolia* leaf had MIC values ranging from 0.031 to 0.98 mg/ml. The extract gave MIC values of 0.031, 0.24, 0.98 and 0.488 against *S*.ser.Typhi ATCC 43579, *S*.ser.Typhi ATCC 13347, *S*.ser.Typhimurium ATCC 1408 and *S. enterica* ATCC 2162, respectively (Table 3). Methanol and ethyl acetate extracts of *T. diversifolia* exhibited MIC values of 0.031 mg/ml each against *S*.ser.Typhi ATCC 43579 and *S.enterica* ATCC 2162, respectively. These values were not significantly different from ethyl acetate extracts of *W.ugandensis* ($p < 0.0001$). It was noted in our study that clinical *Salmonella* strains were sensitive to all *T. diversifolia* extracts at different MIC values (Table 3). Our present study has demonstrated lower MIC values for *T. diversifolia* extracts against *Salmonella* strains than what Ogundare (2007) reported. According to their report, MIC values of chloroform and methanol extracts of *T. diversifolia* were 6.25 and 3.125 mg/ml, respectively against *S. typhi*. The two extracts however gave MIC values of 6.25 mg/ml each against *P. aeruginosa*.

It was noted from this study that plant extracts tested by microdilution technique showed higher anti-*Salmonella* activity compared to values obtained from disc diffusion technique. *W. ugandensis* extracts showed lower MIC values when determined by microdilution method than by disc diffusion method. Olila et al. (2001) has reported that the paper disc retains the active component and does not allow it to diffuse into Muller Hinton agar. The paper disc is composed of cellulose [b-(1-4) linked glucose monomers]. The many free hydroxyls groups present on each glucose residues renders the surface of hydrophilic (Burgess et al., 1999). Thus, if natural products were cationic, they would be expected to adsorb to the surface of the disc and not diffuse into the medium. Consequently, a cationic polar compound displays a good antibacterial activity, but which is therefore not noticeably antibacterial by paper disc diffusion (Cleidson et al., 2007).

Most of the antibiotics used nowadays have lost their effectiveness due to development of resistant genes in microbes (Davis, 1994; Service, 1995). The antibiotics are sometimes associated with side effects such as hypersensitivity, immune suppression and allergic reaction (Ahmad et al., 1998). More interest is being shown in developing alternative antimicrobial drugs for the treatment of infectious diseases without side effects (Berahou et al., 2007; Salomao et al., 2008). The results of our present study demonstrates anti-*Salmonella* activity of *W. ugandensis* and *T diversifolia* that compared well with ciprofloxacin and chloramphenicol. The results obtained from the nine active plant extracts tested are encouraging. Further work is in progress to isolate and identify the bioactive compound(s) that could be used in the development of safer and cost effective alternative drugs for typhoid fever.

Conclusion

The results of our study showed anti-*Salmonella* activity in extracts from *W. ugandensis* and *T. diversifolia* plants. This activity compared well with that of ciprofloxacin and chloramphenicol. The study provides the basis for use of these plants in the development of drugs for management of typhoid fever.

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Conflict of interests

The author(s) have not declared any conflict of interests.

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Review

Ameliorative potentials of medicinal plants on the pathophysiological complications of diabetes mellitus: A review

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Diabetes mellitus is a metabolic disorder with grievous pathophysiological complications which affect various parts of the body and manifesting in different ways which include acute and chronic neuropathy, nephropathy, gastropathy, retinopathy, micro and macro cardiovascular disorders and erectile dysfunction. Incidence and prevalence of diabetes mellitus is fast becoming high in middle and low income countries where about 80% of people living in those countries depend on orthodox medicine. Numerous and varied reports abound in literature on studies conducted to investigate the ameliorative effects of medicinal plants on various pathophysiological complications of diabetes mellitus. This report therefore presents a review of the effects of medicinal plants on the complications of diabetes mellitus stating the components of the plants responsible for these effects and the possible mechanisms.

Key words: Diabetes mellitus, medicinal plants, diabetic complications, neuropathy, nephropathy, gastropathy, retinopathy, cardiovascular diseases, hyperlipidaemia, erectile dysfunction.

INTRODUCTION

Diabetes mellitus is a metabolic disorder that is marked by elevated blood glucose concentration and excretion of glucose in urine (El-Wakf et al., 2011). The disease occur either because of lack of insulin (a hormone that allows blood glucose to enter the cell of the body to generate energy) or because of the presence of factors that opposes the actions of insulin (Peter, 1993). The result of insufficient action of insulin is an increase in blood glucose concentration higher than 160 mg/dl [8 mmol/L above the normal value of 80 to 120 mg/dl (5.6 mmol/L)] a condition known as hyperglycaemia. The disease is a serious lifelong condition that affects about 8 to 10% of the world's population, out of which about one third do not know they have the disease. International

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Diabetes Federation (2011) reported that 366 million people have diabetes in 2011 and by 2030, this would have risen to 552 million. It was also estimated that diabetes caused 4.6 million deaths in 2011 (International Diabetes Federation, 2011).

The commonest symptom of diabetes is thirst, and it is associated with excessive amount of urine as large amounts of glucose are excreted in the urine (Michael, 1999). Other symptoms of the disease include blurry vision from time to time, feeling tired most of the time, losing weight, very dry skin, sores that are slow to heal, getting more infections than usual, slowing of speech and thought, shaking, sweating, unsteadiness, aggressive behaviour, coma and finally unconsciousness (Michael, 1999).

There are three major types of diabetes mellitus; type 1, type 2 and gestational diabetes (International Diabetes Federation, 2011). Type 1 diabetes is caused by an autoimmune destruction of the insulin – secreting beta cells in the pancreas (Lubert, 1995). It is characterized by a partial or complete loss, of insulin producing beta cells and therefore patients require daily injection of insulin. This type of diabetes usually develops during childhood, adolescence or during early adulthood and affects approximately 5 to 10% of all people with diabetes (Dodda and Ciddi, 2014). International Diabetes Federation (2011) reported that Type 1 diabetes affects 78,000 children annually. Although the disease affects only a small percentage of all people with diabetes, it is associated with a greater prevalence of premature complications and mortality than any other forms of the disease (Harris, 1995).

Type 2 diabetes mellitus is the most common type of diabetes mellitus affecting 90 to 95% of people who develop diabetes and it occurs as a result of loss of insulin responsiveness in its target tissues (El-Wakf et al., 2011). Worldwide, about 120 million people suffering from Type 2 diabetes are able to produce insulin but the liver and muscle cells are resistant to its action and most people with type 2 diabetes find out about their diabetic condition after the age of 40, although the numbers of young people, including teenagers, with type 2 diabetes are growing rapidly (Harris, 1995). Increasing age, obesity, diets rich in high glycemic index foods and physical inactivity are risk factors that may enhance the chances of someone developing type 2 diabetes mellitus.

The major complication of diabetes is the damage to the heart and blood vessels which can cause heart attacks, stroke, and poor circulation. These complications are associated not only with elevated blood glucose, but also elevated blood fat (cholesterol) (Ross, 1993). Cholesterol at elevated concentrations tends to deposit on blood vessels, making them narrower, thereby decreasing the delivery of oxygen and nutrients to tissue and increasing the chance of blood to clot (Shor and Phillips, 1999). The effect of narrowing of the blood vessels is the increase of the risk of developing high blood pressure (Mac Mahon, 2000). Diabetic patients also have an increased risk of eyes disease and the damage to the retina associated with diabetes is the leading cause of blindness in adults under age 65 (Ross, 1993). On the other hand, diabetic nephropathy is an important cause of morbidity and mortality, and is now among the most common causes of end-stage renal failure (Peter, 1993). About 30% of patient with type 1 diabetes have developed diabetic nephropathy after 20 years, but the risk after this time falls to less than 1% percent and from the outset, the risk is not equal in all patients (Abbasi et al., 2000).

PATHOPHYSIOLOGICAL COMPLICATIONS OF DIABETES MELLITUS

Diabetes mellitus is usually accompanied by excessive production of free radicals, and it was recently established that hyperglycaemia induced mitochondrial reactive oxygen species (ROS) production could be a key episode in the progress of diabetic complications (Sayyed et al., 2006). Elevated generation of ROS and the simultaneous decline in antioxidative defence mechanisms observed in diabetic patients could promote the development of complications associated with diabetes mellitus (Sharma et al., 2013). These complications are wide ranging and are due at least in part to chronic elevation of blood glucose levels, which leads to damage of blood vessels (Figure 1). In diabetes, the resulting complications are grouped under “microvascular disease” (due to damage to small blood vessels) and “macrovascular disease” (due to damage to the arteries) (Forbes and Cooper, 2013). Microvascular complications include eye disease or “retinopathy,” kidney disease termed “nephropathy,” and neural damage or “neuropathy,” while the major macrovascular complications include accelerated cardiovascular disease resulting in myocardial infarction and cerebrovascular disease manifesting as strokes (Boon et al., 2006; American Diabetes Association, 2009; Rang et al., 2012). Although the underlying etiology remains controversial, there is also myocardial dysfunction associated with diabetes which appears at least in part to be independent of atherosclerosis. Other chronic complications of diabetes include depression (Nouwen et al., 2011), dementia (Cukierman et al., 2005), and sexual dysfunction (Adeniyi et al., 2011). However, abnormalities of lipoprotein and glucose metabolism are often found in people with diabetes mellitus (Genuth et al., 2003). These abnormalities have been hypothesized to be responsible for the damage to cell membranes which, in turn, results in an elevated production of ROS (Sharma et

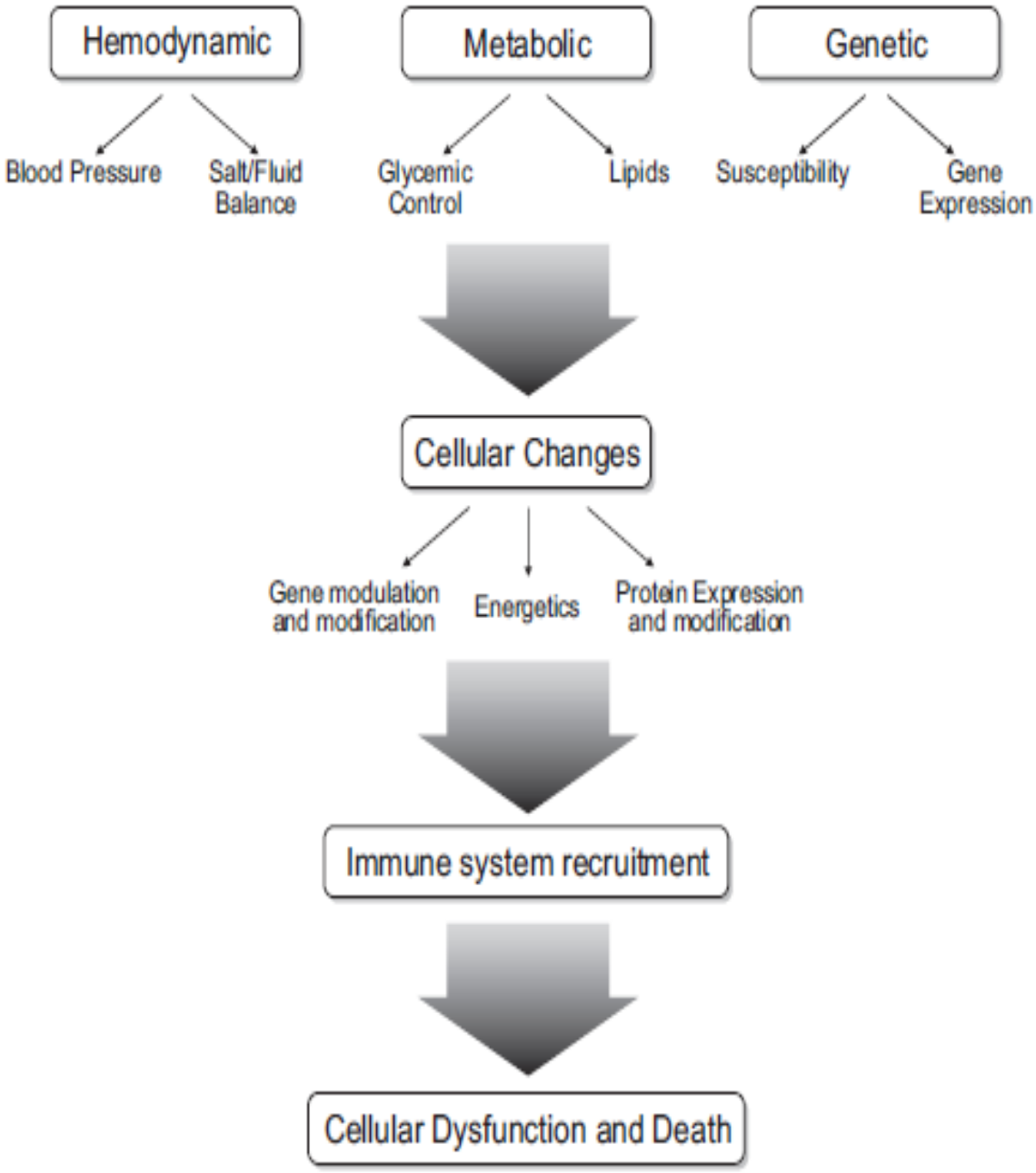

Figure 1. Schematic overview of complications of diabetes mellitus. Source: Forbes and Cooper (2013).

al., 2013).

Mechanisms for the pathophysiological complications of diabetes mellitus

Four molecular mechanisms have been studied for their role(s) in causing diabetic complications and they include: (i) increase in the flux of glucose through polyol pathway, (ii) increased intracellular formation of advanced glycation end-products (AGEs), (iii) activation of protein kinase C (PKC) and (iv) increased flux through the hexosamine pathway (Brownlee, 2001). Among these, polyol pathway (Figure 2) plays an important role in the development of

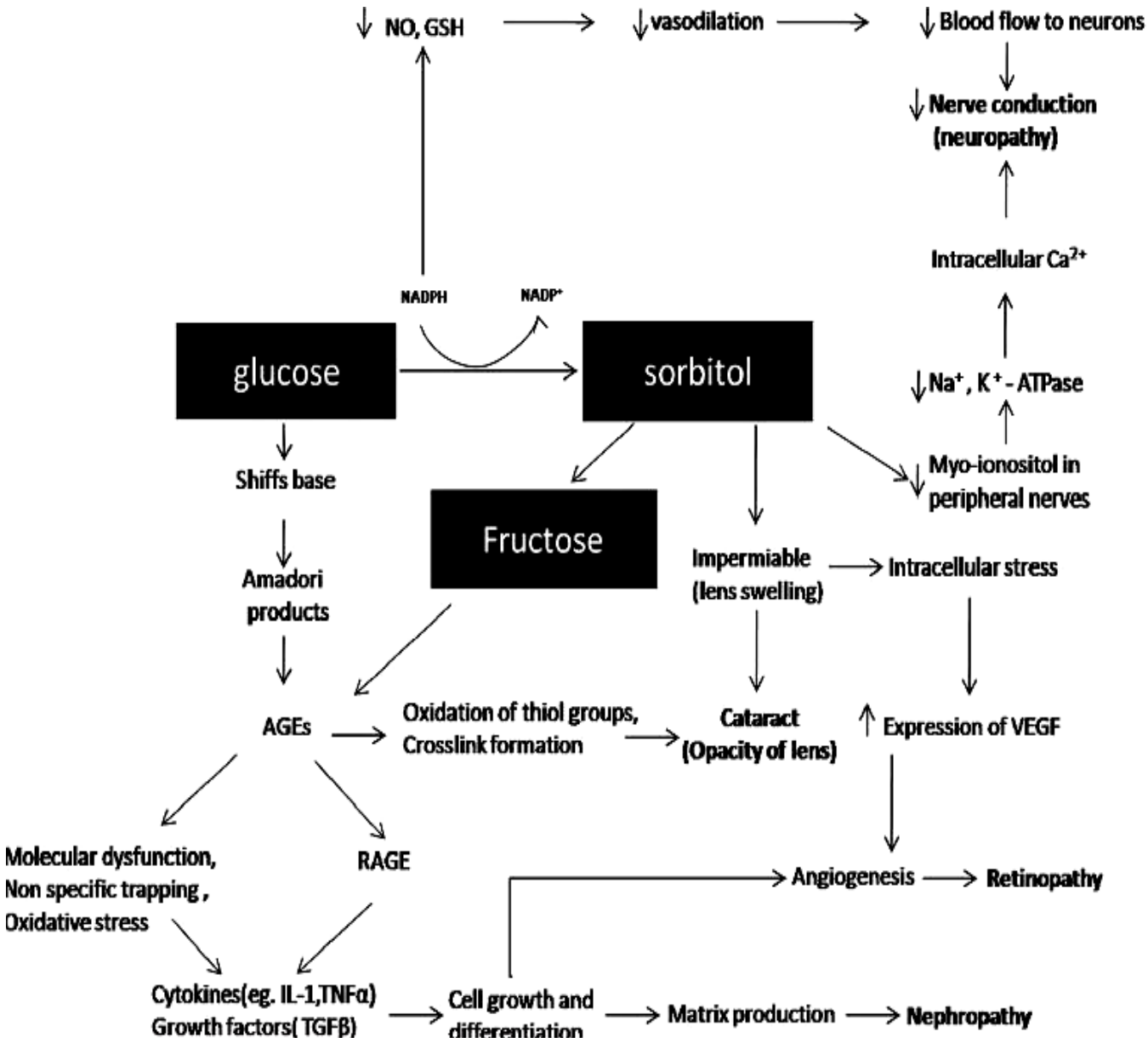

Figure 2. Flow diagram illustrating interplay between the polyol pathway, oxidative stress and diabetic complications. Source: Dodda and Ciddi (2014).

complications in DM (Dodda and Ciddi, 2014). Aldose reductase (AR), which is the first enzyme in the polyol pathway, is a cytosolic, monomeric oxidoreductase enzyme that catalyses the NADPH-dependent reduction of glucose (Wilson et al., 1992). In the polyol pathway, sorbitol is oxidised to fructose by the enzyme sorbitol dehydrogenase, with NAD^+ reduced to NADH (Dodda and Ciddi, 2014). Hyperglycaemia-induced polyol flux leads to increase in sorbitol-induced osmotic stress, decreased Na^+ /K^+-ATPase activity, an increase in cytosolic NADH/NAD^+ ratio and a decrease in cytosolic NADPH (Lee and Chung, 1999). As NADPH is required for regenerating reduced glutathione (GSH), this could induce or exacerbate intracellular oxidative stress leading to changes in respiration, membrane metabolism and oxidative resistance (Garcia et al., 2001).

Although large varieties of drugs have been developed with potent *in vitro* aldose reductase inhibitor (ARI)

activity, yet, very few of them are clinically available because of undesirable side effects and poor pharmacokinetics (Dodda and Ciddi, 2014). This has led to an increased search for newer therapies with mild or no side effects. Herbal medicines are popularly used remedies for many diseases by a vast majority of the world's population and herbal formulations have attained widespread acceptability as anti-diabetics, hepato-protective and lipid lowering agents because of large number of phytochemicals present in plants (Atawodi et al., 2014). Available literatures show that there are more than 400 plant species showing anti-diabetic activity with the possible use in the treatment of DM complications (Grover et al., 2001; Shafi et al., 2012). Herbal formulation alone or in combination with oral hypoglycaemic agents sometimes produces good therapeautic responses in some resistant cases where modern medicines alone have failed (Adeyi et al., 2012). Many herbal products have been prescribed for the management of diabetes mellitus in ancient and recent literature (World Health Organization, 2010). The present review aims at a critical appraisal of the available literature(s) on the use of herbs in the management of diabetic complications.

DIABETIC NEPHROPATHY

Diabetic nephropathy (nephropatia diabetica), also known as Kimmelstiel-Wilson syndrome, nodular diabetic glomerulosclerosis or intercapillary glomerulonephritis, is the condition that occurs when diabetes mellitus causes the kidneys to lose their ability to function properly and it is characterized by high levels of protein usually greater than 0.5 g/24 h in the blood (Shafi et al., 2012; Dodda and Ciddi, 2014). It is a progressive condition culminating into a kidney failure and is now among the most common causes of end-stage renal failure (ESRF) in developed countries (Kim et al., 2009). Pathologically, the first changes seen at the time of microalbuminuria are the thickening of the glomerular basement membrane and accumulation of matrix material in the mesangium. Subsequently, nodular deposits are characteristic, and glomerulosclerosis worsens (heavy proteinuria develops) until glomeruli are progressively lost and renal function deteriorates. Microalbuminuria is an important indicator of risk of developing diabetic nephropathy (Lee, 2003). Progressively increasing albuminuria accompanied by hypertension is much more likely to be due to early diabetic nephropathy (Shafi et al., 2012).

Diabetic nephropathy is one of the most important DM complications affecting 20 to 30% of patients with DM (Ayodele et al., 2004). The onset of diabetic nephropathy is found to be 17 years after the diagnosis of DM (Dodda and Ciddi, 2014). The pathogenesis of DM is attributed to the significant increase of the AR in the glomerulus (Kasajima et al., 2001). Hyperactivation of Aldose reductase in renal cells leads to the generation of AGEs. These AGEs along with the generation of ROS results in the expression and activation of transcription factors like nuclear factor NF-κB and PKC, which are implicated in the pathogenesis of diabetic nephropathy. The AGEs also contribute to the release of proinflammatory cytokines, expression of growth factors and adhesion molecules (Oates and Mylari, 1999). Inhibition of AR in kidney can contribute to the protective effect on diabetic kidney.

THE USE OF MEDICINAL PLANTS FOR THE TREATMENT OF DIABETIC NEPHROPATHY

Some of the medicinal plants reported to be effective in diabetic nephropathy are:

Medicago sativa (Family: Fabaceae; Common name: Alfalfa)

Medicago sativa (Alfalfa) is a perennial flowering plant in the pea family - Fabaceae and is usually cultivated as an important forage crop in many countries of the world. Alfalfa has been used as herbal medicine for over 1,500 years and physicians used young alfalfa leaves to treat disorders related to digestive tract and the kidneys. *M. sativa* leaf extract supplementation has been reported to correct diabetes induced dyslipidaemia, oxidative stress and hepatic renal functions and exerts antihyperglycaemic action as effective as metformin. Leaves of *M. sativa* have been used traditionally in South Africa for treating diabetes in the form of tea (Gray and Flatt, 1997). Owing to its rich source of vitamins and phytoestrogens, it is also used as a food additive in several developed countries.

M. sativa has been reported to possess antihyperglycaemic property and insulin releasing action. The result obtained by Ramachandran et al. (2010) indicates an effective glycaemic control in diabetic animals treated with *M. sativa* extract (MSE) along with a better serum lipid profile. MSE might have other extra pancreatic mechanisms of action in mediating its antihyperglycaemic effect apart from the promotion of increased insulin secretion and action. The extra pancreatic mechanisms of glucose homeostasis might involve enhanced peripheral glucose transport and metabolism as potent as insulin even in absence of insulin, suggesting the competence of MSE to act through terminal pathways of insulin signaling and the mechanisms of MSE dependent glucose homeostasis might also be attributed to its potential to lower glucose

absorption. So also, increased levels in lipid peroxidation, renal and hepatic markers dysfunction were normalized by MSE treatment and the effects were comparable to those seen with metformin treatment. Further, compromised levels of antioxidant enzymes and Na^+- K^+ ATPase in diabetic animals was also brought to normal levels by the powdered leaf of *M. sativa*. MSE also reversed the changes in the levels of serum electrolytes in diabetic animals. These ameliorative changes induced by MSE are more potent than with metformin especially with reference to hepatic dysfunction, providing a basis for the contention that the extract has both hepato and reno protective potentials.

Ginkgo biloba (Family: Ginkgoaceae; Common Name: Maidenhair Tree)

Ginkgos are large trees, normally reaching a height of 20 to 35 m, usually deeply rooted and resistant to wind and snow damage. The tree *Ginkgo biloba* has long been believed to have medicinal properties and its extracts are among the most widely-sold herbal supplements in the world. *G. biloba* extract (GbE), prepared from *G. biloba* leaves, is defined as a complex mixture containing 24% Ginkgo flavoneglycoside (quercetin, kaempferol, and isorhamne) and 6% terpene lactones (ginkgolides and bilobalide) (Hassan and Emam, 2012). It has been used as a therapeutic agent in some cardiovascular and neurological disorders. Zhu et al. (2005) studied the effects of three months oral administration of *G. biloba* leaf in treatment of the renal lesions of early diabetic nephropathy. Indexes such as urinary micro-albumin, alpha-1-microglobulin, immunoglobulin, transferring, retinal binding protein and N-acetyl beta-D-glucosaminidase before and after treatment were compared. The levels of urinary micro-albumin, alpha-1-microglobulin, immunoglobulin, transferring, retinal binding protein and N-acetyl beta-D-glucosaminidase decreased significantly after treatment. The beneficial effects of GbE might be channeled through a combination of one or several mechanisms of action. The combined therapeutic effects are probably greater than that of individual mechanisms and are perhaps the result of the synergistic effects of multiple constituents of the total extract (DeFeudis and Drieu, 2000). The chemical structure of GbE 761, both flavonoid and ginkgolide constituents, is responsible for its remarkable antioxidant/reactive oxygen nitrogen species scavenging activity. The flavonoids preferentially react with hydroxyl radicals and chelate pro-oxidant transition heavy metal ions (Gohil, 2002; Zimmermann et al., 2002). Significant antioxidant activity is consequently one of the most analyzed protective effects of GbE on the central nervous system and the circulatory system. GbE has a number of benefits, including ameliorating hemodynamics, suppressing the platelet-activating factor, scavenging reactive oxygen species and relaxing vascular smooth muscles (Akisü et al., 1998). All of these properties offered a pharmacological foundation for testing GbE for diabetic therapy.

Glycine max (Family: Fabaceae; Common name: Soyabean)

The soyabean is a species of legume native to East Asia and is widely grown for its edible bean which has numerous uses. Soyabean has been researched for its nutritional and health benefits. Lunasin is a peptide found in soyabean and some other cereal grains and has been subject of research focusing on its role in the treatment of cancer, cholesterol, cardiovascular diseases and inflammation. Soyabean have been reported to decrease the progression of diabetic nephropathy by preventing the morphological destruction of the kidney associated with diabetes mellitus (Irritani et al., 1997). Soyabean feeding is known to enhance the conversion of polyunsaturated fatty acids to docosahexaenoic acid. Increased production of this complex lipid has been linked to be beneficial in a variety of ways in the treatment of diseases including renal disease (Shafi et al., 2012). Soyabeans have been shown to reduce urinary albumin excretion and total cholesterol in non-diabetic patients with nephritic syndrome. It may prevent weight loss and morphological disruption of the kidney associated with diabetes mellitus. Soyabean diet improves serum glucose and insulin levels, as well as insulin sensitivity in diabetes. Although the exact mechanism has yet to be elucidated, it is possible that the soluble fiber component of soyabeans may be the most important factor (Anderson et al., 1998). Approximately 15% of the soyabean is composed of insoluble carbohydrates and over 30% of the fiber in soyabeans is of the soluble variety. Moreover, soyabeans are slowly digested and have a low glycaemic index and it contain carbohydrates, fat, protein, vitamins and minerals such as calcium, folic acid and iron (Lavigne et al., 2000) as well as a significant amount of omega-3 fatty acid- alpha-linolenic acid and isoflavones.

Anacardium occidentale (Family: Anacardiaceae; Common name: Cashew Tree)

The cashew tree is a tropical evergreen plant that grows as high as 14 m, but the dwarf cashew growing up to 6 m and many parts of the plants are used in the traditional medicine for the treatment of diseases. In some cases, the seeds are ground into poultice and used in the

treatment of snakebites, the fruits, barks and leaves are used as antifungal, antipyretic and antidiarrheal agents (Akash et al., 2009). Furthermore, Leonard et al. (2006) reported that cashew seed can reduce diabetes induced functional and histological alterations in the kidneys and the hypoglycaemic action of this plant is mostly seen in experimental type I diabetes. Streptozotocin induced diabetes in rats has been reported to be associated with functional and morphological changes in the kidney (Leonard et al., 2006). Albino rats receiving graded doses of hexane extract of this plant (150 and 300 mg/kg/day) showed a significant reduction in blood glucose level, total protein excreted, glycosuria and urea in diabetic rats as reported by Leonard et al. (2006). Leonard et al. (2006) further observed that the histopathological study showed significant reduction in accumulation of mucopolysaccharides in the kidneys of diabetic animals. Phytochemical analysis of the cashew seed has revealed the presence of alkaloids, polyphenols and saponins.

Vernonia amygdalina (Family: Asteraceae; Common Name: Bitter Leaf)

Vernonia amygdalina, a member of the Asteraceae family, is a small shrub that grows in the tropical Africa. *V. amygdalina* typically grows to a height of 2 to 5 m and the leaves are elliptical and up to 20 cm long with a rough bark (Ijeh and Ejike, 2011). African common names of *V. amygdalina* include *grawa* (Amharic), *ewuro* (Yoruba), *etidot* (Ibibio), *onugbu* (Igbo), *ityuna* (Tiv), *oriwo* (Edo), *chusar-doki* (Hausa), *mululuza* (Luganda), *labwori* (Acholi), and *olusia* (Luo) and *Ndolé* (Cameroon) (Egedigwe, 2010; Kokwaro, 2009). *V. amygdalina* is among medicinal plants reported to be used in traditional settings for the management of ailments. Report by Atangwho et al. (2010) showed *V. amygdalina* to restore the damage previously done to the beta cells of the pancreas that is, protective ability of the extracts on the pancreas, as the probable mechanism of action in exerting anti-diabetic action.

Iwara et al. (2013) investigated the effects of combined extracts of *V. amygdalina* (VA) and *Moringa oleifera* (MO) on streptozotocin induced kidney damage in experimental rat models. Significant increases ($p < 0.05$) were observed in K^+, Na^+, Cl^- and urea concentration in groups treated with VA, MO, and MO/VA. This observation may be attributed to the reported presence of bioactive component that are present in these plants and consistent with findings of Musabayane (2012) and Mapanga et al. (2009), which shows that combined leaf extracts of *V. amygdalina* and *M. oleifera* which possess hypoglycemic effect, has the ability to excrete electrolyte in streptozotocin diabetes mellitus, suggesting that this plant may be beneficial in the management of renal dysfunction associated with diabetes mellitus. The result therefore suggests the synergistic effects of the plants in amelioration of nephrotoxicity associated with diabetes mellitus.

Camellia sinensis (Family: Theaceae; Common name: Green tea, Chaay)

Camellia sinensis is the species of plant whose leaves and leaf buds are used to produce the popular beverage tea and it is native to East, South and Southeast Asia, but it is today cultivated across the world in tropical and subtropical regions (Ming, 1992). *C. sinensis* is an evergreen shrub or small tree that is usually trimmed to below 2 m when cultivated for its leaves. The seeds of *C. sinensis* and *Camellia oleifera* can be pressed to yield tea oil, an essential oil that is used for medical and cosmetic purposes. The leaves of the plant however have been used in traditional Chinese medicine and other medical systems to treat asthma (functioning as a bronchodilator), angina pectoris, peripheral vascular disease, and coronary artery disease. Recent medical research on tea (most of which has been on green tea) has revealed various health benefits, including anti-cancer potential, effects on cholesterol levels, antibacterial properties and positive effects for weight loss (Ming, 1992). It is considered to have many positive health benefits due to tea's high levels of catechins, a type of antioxidant. Among other interesting bioactivities, (-)-catechin from *C. sinensis* was shown to act as agonist of PPARgamma, nuclear receptor that is current pharmacological target for the treatment of diabetes type 2 (Wang et al., 2014).

Ribaldo et al. (2009) reported that green tea can prevent diabetes and hypertension-related renal oxidative stress as well as attenuate renal injury. In their experiment, spontaneously hypertensive rats (SHR) with streptozotocin induced diabetes and nondiabetic SHR were treated daily with tap water or freshly prepared green tea. After 12 weeks, the systolic blood pressure did not differ between treated and untreated nondiabetic or diabetic rats. However, body weight was less and glycaemia was greater in diabetic SHR rats than in no diabetic rats. Renal oxidative stress variables were greater in diabetic rats. The oxidative stress parameters were significantly less in rats treated with green tea. These findings suggest that the consumption of green tea may reduce nephropathy in diabetic hypertensive patients.

Cinnamomum zeylanicum (Family: Lauracaeae; Common name: Dalchini)

Cinnamomum zeylanicum (new botanical name:

Cinnamomum verum) trees can grow up to a height of 10 to 15 m and the leaves are ovate-oblong in shape, with flowers that are greenish in colour which are arranged in panicles. In addition to its culinary uses, in native Ayurvedic medicine Cinnamon is considered a remedy for respiratory, digestive and gynaecological ailments. Almost every part of the cinnamon tree including the bark, leaves, flowers, fruits and roots, has some medicinal or culinary use. The volatile oils obtained from the bark, leaf and root barks vary significantly in chemical composition, which suggests that they might vary in their pharmacological effects as well (Shen et al., 2002). The different parts of the plant possess the same array of hydrocarbons in varying proportions, with primary constituents such as; cinnamaldehyde (bark), eugenol (leaf) and camphor (root) (Gruenwald et al., 2010). Thus cinnamon offers an array of different oils with diverse characteristics, each of which determines its' value to the different industries. For example the root which has camphor as the main constitute, has minimal commercial value unlike the leaf and bark (Paranagama et al., 2010). It is this chemical diversity that is likely to be the reason for the wide-variety of medicinal benefits observed with cinnamon. The ameliorative effect of the cinnamon oil upon early stage diabetic nephropathy due to its antioxidant and antidiabetic effect has been studied against alloxan (150 mg/kg intraperitonally) induced diabetic nephropathy by Mishra et al. (2010).

Histological studies of the kidney revealed the protecttive effect of cinnamon oil by reducing the glomerular expansion, eradicating hyaline casts and decreasing the tubular dilatations. The results indicated that the volatile oil from cinnamon contained more than 98% cinnamaldehyde and that it confers dose-dependent significant protection against alloxan induced renal damage. The maximum decrease in fasting blood glucose has been achieved at the dose of 20mg/kg (Mishra et al., 2010).

Curcuma longa (Family: Zingiberaceae; Common name: Turmeric)

Turmeric is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae and it is native in southeast India (Chan et al., 2009). The most important chemical components of turmeric are a group of compounds called curcuminoids, which include curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin of which the best studied compound is curcumin, which constitutes 3.14% (on average) of powdered turmeric (Tayyem et al., 2006). In addition, there are other important volatile oils such as turmerone, atlantone, and zingiberene and some general constituents such as sugars, proteins, and resins (Nagpal and Sood, 2013). In India, turmeric has been used traditionally for thousands of years as a remedy for stomach and liver ailments, as well as topically to heal sores, basically for its supposed antimicrobial property (Chaturvedi, 2009). In the Siddha system (since c. 1900 BCE) turmeric was a medicine for a range of diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders. A fresh juice is commonly used in many skin conditions, including eczema, chicken pox, shingles, allergy, and scabies (Khalsa, 2013). The active compound curcumin is believed to have a wide range of biological effects including anti-inflammatory, antioxidant, antitumour, antidiabetic, antibacterial, and antiviral activities, which indicate potential in clinical medicine (Aggarwal et al., 2007). Chronic treatment with curcumin obtained from *Curcuma longa* significantly attenuates both renal dysfunction and oxidative stress in streptozotocin induced diabetic rats.

The results confirmed evidence of oxidative stress in diabetic nephropathy and point towards the possible anti-oxidative mechanism being responsible for the nephroprotective action of curcumin (Sharma et al., 2006).

Brassica oleracea (Family: Brassicaceae; Common name: Red Cabbage)

B. oleracea has become established as an important human food crop plant, used because of its large food reserves, which are stored over the winter in its leaves. It is rich in essential nutrients including vitamin C and a diet rich in cruciferous vegetables (for example, cabbage, broccoli, cauliflower) is linked to a reduced risk of several human cancers (Verhoeven et al., 1996). It is mainly used as a vegetable and it has anti-oxidant and antihyperglycaemic activities (Shafi et al., 2012). Main constituents are the isothiocyanates and anthocyanins, reduces oxidative diabetic nephropathy (Evans et al., 2002). It contains anthocyanin pigments that are described as free radical scavenging and antioxidant agents. Its extract contains vitamins A, B and C all of which have protective roles against oxidative damage (Fowke et al., 2003). It also contains substantial quantities of isothiocyanates some of which are very potent anti-oxidants. Daily ingestion of red cabbage polar extract (g/kg body weight) ameliorates oxidative stress and diabetic nephropathy (Shafi et al., 2012).

Ganoderma lucidum (Family: Ganodermataceae; Common name: Lingzhi Mushroom)

Lingzhi is a mushroom that is soft (when fresh), corky

and flat, with a conspicuous red-varnished, kidney-shaped cap and, depending on specimen age, white to dull brown pores underneath (Arora, 1986). *Ganoderma lucidum*, an oriental fungus has a long history of use for promoting health and longevity in China, Japan and other Asian countries. In Chinese, the name lingzhi represents a combination of spiritual potency and essence of immortality, and is regarded as the "herb of spiritual potency," symbolizing success, well-being, divine power and longevity. Among cultivated mushrooms, *G. lucidum* is unique in that its pharmaceutical rather than nutritional value is paramount. The specific applications and attributed health benefits of lingzhi include control of blood glucose levels, modulation of the immune system, hepatoprotection, bacteriostasis and more. Various polysaccharides have been extracted from the fruit body, spores, and mycelia of lingzhi; they are produced by fungal mycelia cultured in fermenters and can differ in their sugar and peptide compositions and molecular weight (for example, ganoderans A, B, and C). *G. lucidum* polysaccharides (GL-PSs) are reported by Bao et al. (2001) and Wachtel-Galor et al. (2004) to exhibit a broad range of bioactivities, including anti-inflammatory, hypoglycemic, antiulcer, antitumorigenic and immunostimulating effects.

The effects of *G. lucidum* polysaccharide on renal complication in streptozotocin induced diabetic mice were studied by He et al. (2006). From their findings, extract of *G. lucidum* was able to reduce the serum creatinine and blood urea nitrogen levels and urinary albumin excretion compared with diabetic model mice in a dose dependent manner. Increasing serum glucose and triglyceride levels in diabetic mice could also be lowered by *G. lucidum* polysaccharide. It has a capacity to improve the metabolic abnormalities of diabetic mice and prevent or delay the progression of diabetic renal complications (He et al., 2006).

Indigofera tinctoria (Family: Fabaceae; Common name: True Indigo)

True indigo is a shrub, one to two metres high and it may be an annual, biennial, or perennial, depending on the climate in which it is grown. It has light green pinnate leaves and sheafs of pink or violet flowers. The herb is widely used in the Indian system of medicine for epilepsy, nervous disorders, bronchitis and liver ailments (Singh et al., 2001). Extensive research of the last few decades has revealed that the herbal extract is used as an anticardiovascular agent (Narender et al., 2006). It has been used so as to protect rat against hepatoxicity induced by CCl_4 and as a liver antioxidant (Sreepriya et al., 2001). The family of bis-indoles known generically as indirubins is the main constituents of *I. tinctoria;* a product from Chinese material medica used to treat myelogenous leukemia and possesses cytotoxic activity (Cragg and David, 2005). The study carried out by Bangar and Saralay (2011) shows that the extract from leaves improved renal creatinine clearance and reduced renal total protein loss demonstrating nephroprotective properties. The organ to body weight ratio studies carried out showed pancreas and liver specific effects of *I. tinctoria* leaves. These results were also supported by histo-pathological studies. It was concluded from the studies that alcoholic extract of leaves in long-term treatment may be beneficial in the management of type-1 and type-2 diabetes.

Panax quinquefolius (Family: Araliaceae; Common name: American Ginseng)

American ginseng (*Panax quinquefolius*) is a perennial herbaceous plant, commonly used in Chinese or herbal medicine and the effects of American ginseng and heat-processed American ginseng on diabetic renal damage using streptozotocin induced diabetes was studied by Kim et al. (2009). The diabetic rats have shown a loss of body weight gain and increase in kidney weight, food intake, and urine volume, whereas the oral administration of heat processed American ginseng at a dose of 100 mg/kg body weight per day for 20 days attenuated these diabetes- induced physiological abnormalities. Among the renal function parameters, the elevated urinary protein levels in diabetic control rats were significantly decreased by the American ginseng or heat processed American ginseng administration, and the decreased creatinine clearance level was significantly increased in rats administered with heat processed American ginseng. These findings indicated that heat processed American ginseng may have beneficial effect on pathological conditions associated with diabetic nephropathy.

DIABETIC NEUROPATHY

Diabetic neuropathy (DN) is a secondary microvascular complications of diabetes mellitus causing damages to the nerves and is characterized by fall in nerve conduction velocity, severe pain, impaired sensation and degeneration of nerve fibres (Anjaneyulu and Chopra, 2004). It is also characterized by hyper responsiveness to pain typically originating in the extremities, followed by progressive loss of neuronal function in a distal to proximal gradient (Feldman et al., 1997).

There are many different diabetic neuropathies involving different nerve types, which are mainly featured by diffuse or focal damage to peripheral somatic or

autonomic nerve fibres. Hence, DN can be classified into diffuse and focal neuropathies with diffuse neuropathy being more common, chronic and progressive, whereas focal neuropathies are less common and acute in nature (Anjaneyulu and Chopra, 2004). However, all these neuropathies are thought to occur from hyperglycaemia-induced damage to nerve cells and from neuronal ischaemia resulting from hyperglycaemia-induced changes (Edwards et al., 2008).

The exact etiopathogenesis of DN is multifactorial and involves various factors such as hyperglycaemia, neuronal loss, alterations in neurotransmitters and growth factors etc (Anjaneyulu and Chopra, 2004). Other mechanisms of DN include insulin deficiency, oxidative stress, nitrosative stress, ischaemia, osmolyte accumulation, neurotropic factor deficiency, autoimmune nerve destruction, alterations in cellular signaling pathways and gene expression of protein (Kannan, 2000). Increase in sorbitol concentrations by polyol pathway can lead to cellular injury and decrease of myo-ionositol in the peripheral nerves and thereby leading to decrease in Na^+/K^+-ATPase activity, which is essential for nerve conduction (Oka and Kato, 2001). Moreover, decreased NADPH results in decreased nitric oxide and reduced GSH production resulting in decreased vasodilatation and increased ROS production and oxidative damage. Thus, substances containing aldose reductase inhibitors are likely to ameliorate the development of diabetic neuropathy.

Although, the precise records of neuropathic pain sufferers are not available, it is estimated that more than one million people worldwide are suffering from this condition (Hall et al., 2006). At present there is no definitive course of treatment available for diabetic neuropathy, as it is not clearly understood. Tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRI), anticonvulsants, opioids and topical capsaicin have been used in the management of painful diabetic neuropathy.

The use of all these classes of drugs is restricted by their cost and side effects. Furthermore, these are only moderately effective, produce potential toxicity and develop tolerance, so the need for newer, better tolerated and efficacious treatment is in high demand (Ziegler, 2008). There is now enormous support that herbal drugs may be helpful in the cure and control of neuropathy and this may translate directly or indirectly to the management of diabetic complications.

The use of medical plants in the treatment of diabetic neuropathy

Some of the medicinal plants with potential use in the treatment of DN are described:

Cleome viscosa (Family: Capparadiceae; common name: Dog mustard)

Cleome viscosa is an annual, sticky herb with yellow flowers and lengthy slender pods containing seeds which bear a resemblance to those of mustard with strong penetrating odour (Parimala Devi et al., 2004). The plant contains lignans, flavonoids, saponins, ascorbic acid and polyunsaturated fatty acid, and some other chemical constituents such as glucosinolates, cleomeolide, Stigmasta-5, 24 (28)-diene-3β-*O*-α-L-rhamnoside, kaempferide-3-glucuronide and naringenin glycoside (Sudhakar et al., 2006). Traditionally, herbal formulations of *C. viscosa* are used as laxative, anti-helminthic, stomachic, diuretic and hypoglycemic agents (Rukmini, 1978; Yaniv et al., 1987; Gupta et al., 2009). Rao et al. (2014) investigated the neuroprotective effect of ethanolic extract of *C. viscosa* (EECV) against streptozotocin induced diabetic neuropathy in Wistar rats. Intraperitoneal injection of streptozotozin resulted in significant increase in thermal hyperalgesia and hyperlipidaemia after four weeks. Antioxidant enzyme (superoxide dismutase (SOD), glutathione (GSH) and catalase) levels were reduced and malondialdehyde (MDA) level was increased significantly in diabetic rats as compared to the vehicle control rats. Four weeks of treatment with EECV (100, 200 and 400 mg/kg) attenuated the level of nociceptive threshold significantly ($p < 0.05$) and dose dependently, thus suggesting the role of ROS mediated oxidative stress in nociceptive changes in STZ induced diabetic rats. It also significantly ($p < 0.05$) decreased the elevated levels of lipids, lipid peroxidation and oxidative stress and this was also dose dependent. *C. viscosa* is already proved to have antioxidant properties, and this may trim down the susceptibility of lipids to oxidation and cause the membrane lipids stabilization, thus reducing oxidative stress. The study of Rao et al. (2014) therefore provides investigational evidence of the protective effect of EECV on nociception, hyperlipidaemia and oxidative stress in streptozotocin induced diabetic neuropathy.

Aegle marmelos (Family: Rutaceae; common name: Stone apple/golden apple/wood apple/Bengal quince/bael)

The tree grows throughout deciduous forest of India and ripen fruits are commonly used for delicacy and are also widely used in Indian Ayurvedic medicine for the treatment of diabetes mellitus (Kamalakannan and Stanley, 2003). *A. marmelos* is well known for its antihyperglycemic, analgesic, anti-inflammatory and anti-oxidant properties (Sabu and Kuttan, 2004). Bhatti et al.

(2012) investigated the effect of *A. marmelos* leaf extract (AME) on hyperalgesia in alloxan-diabetic rats. The diabetic animals exhibited first symptoms of hyperalgesia from 7th day of alloxan injection and maximal hyperalgesia was observed between 12th to 14th day of inducing diabetes. The diabetic animals were treated with vehicle (diabetic control), varying doses of AME (25, 50, 100, 200 and 400 mg kg^{-1}), fluoxetine (20 mg kg^{-1}), propanolol (30 mg kg^{-1}) followed by AME (100 mg kg^{-1}) and yohimbine (2 mg kg^{-1}) followed by AME (100 mg kg^{-1}) from 3rd to 14th day of induction of diabetes. AME was found to increase the paw licking and tail flicking latency ($p < 0.05$) as compared to the vehicle treated diabetic controls. The effect of AME was found to be dose dependent with maximum dose dependent increase observed at a dose of 100 mg kg^{-1}. Aegeline found in the alcohol extract of *A. marmelos* has been proposed to have a structural similarity to adrenergic receptor ligands and it has been generally accepted that both α and β adrenergic receptors allocated on the membrane surface of beta cells of pancreas regulate the insulin release (Narener et al., 2007). The α_2 adrenergic receptors are proposed to be the major adrenergic receptor involved in the modulation of insulin release in pancreatic beta cells (Nakadate et al., 1981). The pretreatment with propanolol did not alter the *perse* effect of AME. On the other hand administration of yohimbine prior to AME was found to attenuate the protective effect of AME. Also, the antinociceptive effect of *A. marmelos* may involve interplay between adrenergic neurons and other neurotransmitters. From the findings, Bhatti et al. (2012) tentatively concluded that AME provides protection against alloxan induced diabetic neuropathy in rats and this effect might be mediated via the autonomic nervous system**.**

Momordica charantia **(Family: Cucurbitaceae; common name: Bitter melon/bittergourd/balsam pear)**

Bittergourd is one of the popular herbs found in Nigeria and is known in some tribes of Nigeria as Ejirin wewe (Yoruba), Ndeme (Igbo) and Garafun (Hausa) (Komolafe et al., 2012). Various parts of *M. charantia* such as the seed, fruits and even the whole plants has been reported to have beneficial effects in the prevention and treatment of DM in individuals with non-insulin dependent diabetes (Platel and Srinivasan, 1997). It has hypoglycaemic properties as it significantly suppressed the rise in blood glucose concentrations in albino rats (Nicholas et al., 2006).

The first clinical study into the influence of the fresh juice of bittergourd on the management of DM was Akhtar et al. (1981). These findings suggested that the intervention would effectively treat all symptoms of diabetes including polyuria, polydipsia and polyphagia. Bitter melon contains an array of biologically active plant chemicals including triterpenes, proteins and steroids. In numerous studies, at least three different groups of constituents found in all parts of bitter melon have clinically demonstrated hypoglycemic properties or other actions of potential benefits against DM and these chemical include a mixture of steroidal saponins known as charantins, insulin-like peptides and alkaloids (Tan et al., 2007).

The hypoglycemic effect is more pronounced in the fruits of bitter melon where these chemicals are found in greater abundance (Komolafe et al., 2012). However, administration of a *M. charantia* fraction with potent ARI activity in diabetic rats has been reported to lead to a slight increase in myelinated fibre area, even though, the mechanism for this beneficial effect of *M. charantia* on the structural abnormalities of peripheral nerves in experimental DM was not established (Celia et al., 2003). Furthermore, Komolafe et al. (2012) investigated the effects of *M. charantia* on the histological changes of the left ventricle of the heart in STZ-induced diabetic wister rats.

Histologically, there was evidence of architectural alteration in the myocardium of diabetic animals and the effects were abrogated with the administration of *M. charantia* extract and glimepiride for four weeks and this observation may have been possible due to a reduction in blood glucose level leading to enhanced peripheral glucose utilization. Tripathi and Chandra (2009) also reported that *M. charantia* extracts potentiate the insulin effect by rejuvenation of damaged beta cells. So also, distribution of elastic fibres were observed to be sparsely distributed as evident by the staining intensity in the left ventricle of diabetic rats when compared with the control group and this observation suggests a reduction in the tensile strength and elasticity of the heart. Administration of *M. charantia* and glimperide gradually restored the integrity of these fibres thereby reducing the susceptibility to cardiovascular complications. All these evidences suggest cardio-protective effects of *M. charantia* against anatomical derangements observed in the diabetic group, thus, the findings of Komolafe et al. (2012) showed that the methanolic extract of *M. charanta* has a promising ameliorative effects on the associated cardiac complications implicated in the STZ induced diabetes in rats.

Moringa oleifera **(Family: Moringaceae, common name: Drumstick tree)**

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae, which is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. It is used in traditional folklore for

treating many ailments such as asthma, spasm, enlarged liver and spleen, infection and nervous debility, ulcer, inflammation and for wound healing (Mishra et al., 2011; Promkum et al., 2010). This tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, calcium, iron, vitamin C, and carotenoids suitable for utilization in many of the so-called “developing” regions of the world where undernourishment is a major concern. Studies have also shown that the extract of *M. oleifera* leaves also possesses antidiabetic and antioxidant activities (Pari et al., 2007; Jaiswal et al., 2009). Khongrum et al. (2012) investigated the activity of leaf extract of *M. oleifera* in improving neuropathic pain induced by diabetic condition. Diabetic (induced with STZ) rats were induced with neuropathic pain by constricting the right sciatic nerve (CCI) permanently.

Thereafter, all rats were administered the extract of *M. oleifera* leaves at doses of 100, 200 and 300 mg/kg BW once daily in a period of 21 days. The analgesic effect of the plant extract was evaluated using Von Frey filament and hot plate tests every 3 days after CCI throughout 21-day experimental period.

At the end of the experiment, the alteration of oxidative damage markers including malondialdehyde (MDA) level and the activities of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione peroxidise (GSH-Px) in the injured sciatic nerve were also evaluated. The results obtained showed that rats subjected to *M. oleifera* leaves extract at doses of 100 and 200 mg/kg BW significantly reversed the decreased withdrawal threshold intensity and withdrawal latency in Von Frey filament and hot plate tests, respectively. In addition, rats subjected to the medium dose extract also reversed the decreased activities of SOD and GSH-Px and the elevation of MDA level in the injured nerve. Therefore, the decreased MDA level in rats subjected to *M. oleifera* leaves extract at low dose might be due to either the decreased oxidative stress formation or due to the enhanced non-enzymatic scavenging activity. In addition, the possible underlying mechanism contributing the important role on the analgesic effect of *M. oleifera* leaves extract may be attributed not only to the decreased oxidative stress damage but also to other mechanisms.

Based on the previous finding that both the inhibition of calcium channel and cyclo-oxygenase 2 (COX-2) are also playing the role on the hyperalgesia and allodynia in neuropathic pain condition (Muthuraman and Singh, 2011), it was suggested that the mechanism just mentioned may also contribute to the role in the analgesic effect of *M. oleifera* extract especially at a low dose concentration (100 mg/kg). The results obtained therefore suggest that *M. oleifera* leaves extract can attenuate neuropathic pain in diabetic condition.

***Coccinia indica* (Family: Curcubitaceae; common name: Little gourd)**

Coccinia indica has been used extensively in Ayurvedic and Unani practice in the Indian subcontinent (Kohli and Kumar, 2014). Ivy plant has been used in traditional medicine as a household remedy for various diseases, including biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders. For the last few decades, some extensive work has been done to establish the biological activities and pharmacological actions of Ivy Gourd and its extracts. Polyprenol (C60- polyprenol (1)) is the main yellow bioactive component of Ivy Gourd and has been shown to have antidyslipidaemic of biological actions. Kohli and Kumar (2014) investigated the use of combined *C. indica* with low dose of acarbose treating diabetic neuropathic pain as well as restoring blood glucose level and antioxidant status. The essence of administering low dose of acarbose to the experimental animals was to reduce hypoglycemic effect due to DM induction. Diabetic rats treated with C*. indica* alone and in combination with low dose of acarbose produced significant decrease in the blood glucose level after 7 weeks of treatment. Blood glucose lowering activity may be due to the inhibition of intestinal glucose uptake, insulin secreting property, insulinotropic activity of the component present in the extract.

Hypoglycaemic action of *C. indica* could be due to its ability to potentiate the insulin effect of plasma by increasing the pancreatic secretion of insulin from the existing beta cells (Venkateswaran and Pari, 2002; Shakya, 2008). Treatment with combined *C. indica* leaf and acarbose showed a significant decrease in blood glucose levels of the diabetic rats. Oxidative induced damage to the beta cells can be prevented by herbal therapy due to potential antioxidant property. It has been reported that saponins, cardenolides, quercetin, terpenoids, flavonoids and polyphenols present in *C. indica* leaf posses the antioxidant, anti-inflammatory properties (Pari and Venkateswaran, 2003). *C. indica* leaf extract decreased the thiobarbituric acid reactive substances (TBARS) level and increased the SOD and CAT level in the diabetes neuropathic rats. Quercetin proved to be capable of stimulating beta cell and inducing insulin secretion. Terpenoids were also found to be responsible for antidiabetic activity of the *C. indica* leaf extract. Untreated diabetic rats showed significant hypersensitivity towards thermal stimuli when compared with normal control.

In preventive therapy the acarbose, CI and combinations produced increase tail flick latency in tail immersion test and paw withdrawal in hot plate test. The increased tail flick latency may be due to their property to control blood glucose level and its analgesic and inflame-matory property (Kamble et al., 1998). Histopathological

studies proved that there is no damage in the sciatic nerve of the groups treated with the ethanolic extract of *C. indica* and its combination with low dose of acarbose. Hence based on the result, it is concluded that ethanolic extract of *C. indica* leaf with low dose of acarbose can be used as antinociceptive agent due to its antidiabetic and antioxidant property.

Astragalus membranaceus Bunge (Fisch.) (Family: Leguminosae; common name: Astragalus)

Astragalus membranaceus is an important herb commonly used in Chinese homeopathic homes. It has been used in a wide variety of herbal blends and 'natural' remedies. *A. membranaceus* has been researched for its cardioprotective, anti-inflammatory, and longevity effects. The flavonoid content of *A. membranaceus* may also contribute to its cardioprotective effects. Its poly-saccharide content also protects the heart because it is a potent anti-inflammatory agent, and it is able to reduce cholesterol levels. The main mechanism of *A. membranaceus* is a result of its active ingredients. The protective mechanism of AGS-IV, a new glycoside of cycloartane-type triterpene isolated from the root of *A. membranaceus* (Fisch.) decreases the blood glucose concentration and HbAlC levels, and increases plasma insulin levels. AGS-IV increases the activity of glutathione peroxidase in nerves, depress the activation of aldose reductase in erythrocytes, and decreases the accumulation of advanced glycation end products in both nerves and erythrocytes. Moreover, it elevates Na^+, K^+-ATPase activity in both the nerves and erythrocytes of diabetic rats. These results indicate that AGS-IV exerts protective effects against the progression of peripheral neuropathy in STZ-induced diabetes in rats through several interrelated mechanisms (Yu et al., 2006).

DIABETIC RETINOPATHY

Diabetic retinopathy (DR) is the most common ocular complication in DM and is an important cause of preventable blindness. DR is broadly classified as nonproliferative DR involving intraretinal microvascular changes and proliferative DR involving the formation of new vessels or fibrous tissue or both on the retina. DR primarily effects the microvascular circulation of the retina. The factors leading to these changes are thickening of basement membrane of the capillary wall, increased platelet stickiness and changes in red blood cells (RBCs) resulting in sluggish microvascular circulation and biochemical changes in the form of activation of polyol pathway resulting in tissue damage. Since the retinal ganglionic cells and endothelial cells are endowed with aldose reductase (AR) enzyme, these cells are more prone to damage caused by the activation of polyol pathway leading to DR. Early development of cataract of lens is due to the increased rate of sorbitol formation, caused by hyperglycaemia (Eshrat and Hussain, 2002). Glycosylation of retinal proteins and retinal micro vascular abnormalities lead to retinopathy and blindness (Kelvin and Moss, 1992). Glycosylation of lysine residues of lens proteins also causes cataract formation.

THE USE OF MEDICINAL PLANTS IN THE TREATMENT OF DIABETIC RETINOPATHY

A variety of plant preparations have been mentioned in Ayurveda and other indigenous systems of medicine, which are claimed to be useful in diabetes mellitus and their complications (Shukla et al., 2000) and they include the following:

Azadirachta indica (Family: Meliaceae; common name: Neem)

Neem is a fast-growing tree that can reach a height of 15 to 20 m (49 to 66 ft), rarely to 35 to 40 m (115 to 131 ft). It is an evergreen tree, but in severe drought, it may shed most or nearly all of its leaves. *A. indica* Juss leaves have been reputed to possess cardiovascular, antimicrobial, immunomodulatory, hypoglycemic and a number of other effects (Chattopadhyay, 1996). A bitter principle, *nimbi din*, isolated from seeds of neem tree was reported to be effective in reducing fasting blood glucose at a dose of 200 mg/kg in alloxan diabetic rabbits (Sonia and Srinivasan, 1999). Aqueous extract of tender neem leaves was also reported to reduce blood glucose and this effect was due to its ability to block the actions of epinephrine on glycogenolysis and peripheral utilization of glucose (Chattopadhyay et al., 2000). Eshrat and Hussain (2002) carried out a study to investigate the effect of oral feeding of aqueous extract of fresh leaves of *A. indica* in streptozotocin induced diabetes and its associated retinopathy in rats. The streptozotocin injected rats developed not only diabetes as indicated by increased fasting blood glucose values, but also showed external signs of retinopathy by 10 days. The eyes of diabetic rats looked opaque even from outside. After treatment for 16 weeks, the eyes of the treated group of rats appeared normal from outside. Photograph of the retina indicated that the laser spots has disappeared, therefore pointing out that the treatment reversed the changes in the eye, that is, abnormal changes of retinopathy almost disappeared. Treatment of the diabetic rats with aqueous extract of leaves of *A. indica* at

a dose of 250 mg/kg body weight for 16 weeks resulted in gradual but significant fall in blood glucose and improvement in total serum, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol and triacylglycerol which increased in diabetic rats. Thus, the present study indicates that treatment with water extract of neem leaves has favorable effect not only on blood glucose and glucose tolerance but also on lipid profile and body weight. Another interesting feature of this plant product is that it completely reversed the abnormal change in retina and inflammation of paws. This shows the promising effects of *A. indica* being a useful anti-diabetic agent and its ability to reverse complications of retinopathy and cardiovascular changes in diabetes.

Tinospora cordifolia (Family: Menispermaceae; common name: Giloe)

Tinospora cordifolia is an Ayurvedic plant and it is distributed throughout tropical Indian subcontinent, Sri Lanka and China, ascending to an altitude of 300 m (Sharma et al., 2014). The plant is commonly used in rheumatism, urinary disease, dyspepsia, general debility, syphilis, skin diseases, bronchitis, spermatorrhea and impotence. The arabino-galactan polysaccharide isolated from *T. cordifolia* has been reported to have an anti-oxidant effect in normal animals as well as in diabetic animals. It also reported to be used in treatment of diabetic complications like retinopathy and neuropathy (Agrawal et al., 2012; Nadig et al., 2012). The phytoconstituents of *T. cordifolia* including alkaloids are known to have hypoglycemic effect (Grover et al., 2000; Patel and Mishra, 2011; Patel and Mishra, 2012; Sangeetha et al., 2013). Various chemical constituents have been isolated from different part of *T. cordifolia* and they belong to different classes such as flavanoids, saponin, glycosides, steroids, alkaloids, sesquiterpenoid, polysaccharides diterpenoids lactones, phenolics and aliphatic compounds (Jasuja et al., 2014). Three major groups of compounds; protoberberine, alkaloids, terpenoids and polysaccharides are considered as putative active constituents of *T. cordifolia* (Jasuja et al., 2014).

DIABETIC INDUCED HEPATOPATHY

Diabetic hepatopathy also known as glycogen hepatopathy is a disease of the liver which causes lesions to develop on the liver. It is associated with diabetes mellitus, and for unknown reasons, this type of liver disease is also associated with lesions on the skin. One of the possibilities may be a link to metabolic system and a change in the organ systems. Glycogen hepatopathy (GH) has been characterized as a pathologic overloading of hepatocytes with glycogen that is associated with poorly controlled type 1 diabetes mellitus (Fridell et al., 2007). Clinically, it presents with a spectrum of clinical signs and symptoms, including abdominal discomfort, tender hepatomegaly and elevated transaminases.

THE USE OF MEDICINAL PLANTS IN THE TREATMENT OF DIABETIC INDUCED HEPATOPATHY

Medicinal plants are good source of natural antioxidants believed to exert their effects by reducing the formation of the final active metabolite of the drug-induced systems or by scavenging the reactive molecular species to prevent them from reaching a target site (Kaleem et al., 2005). It has been documented that several medicinal plants have great hypoglycemic effects which is mainly associated with a significant alteration in the activity of liver hexokinase (Bopanna et al., 1997) and glucokinase (Kumari et al., 1995). In addition, Mansour et al. (2002) demonstrated that the administration of several herb extracts could restore the changes in the activities of serum liver enzymes, like transaminases (AST and ALT) as well as alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) of diabetic rats**.** Aminotransferases (AST and ALT) mediate the catalysis of aminotransfer reactions and they are markers for clinical diagnosis of liver injury (Li et al., 2007). ALP is a hydrolase enzyme responsible for removing phosphate group from nucleotides and proteins, it is produced primarily in liver and brain (Han et al., 2006), and it is a marker of hepatic functions (Yoo et al., 2008). LDH is a general indicator of acute or chronic hepatic damage, as well as determining organ, cell and tissue condition (Yoo et al., 2008). Some of the herbs that can be used in the treatment of diabetic induced hepatopathy are described.

Cichorium spp (Family: Asteraceae; common name: Sun flower)

Cichorium spp is a bushy perennial plant and the rhizome is light yellow outside, white from within, containing milky, bitter juice. *Cichorium endivia* is a very highly nutritious plant, with a high content of dietary fibres, potassium and vitamin C (Kopeck, 1998). The antibacterial, antimalarial, cytotoxic, antidiabetic, no-mutagenic and other activities of chicory were evaluated previously by Petrovic et al. (2004). Earlier studies have reported that ethanolic extract of *Cichorium intybus* has antidiabetic and hypolipidaemic activities in STZ-induced diabetic rats (Pushparaj et al., 2007). Similarly, Upur et al. (2009) reported that *Cichorium glandulosum* extract can reduce serum AST, ALT and ALP activities after CCl_4 and

galactosamine administration, which induced acute hepatotoxicity in mice, and this suggest that *C. glandulosum* is a potent hepatoprotective agent that could protect liver against the acute injury and this ability might be attributed to its antioxidant potential. Furthermore, from the research carried out by Kamel et al. (2011), the leaf powder of *C. endivia* produced significant hepatoprotective effects by decreasing the activities of serum aminotransferases (AST and ALT), ALP, LDH and liver malondialdehyde (MDA) level as well as liver superoxide dismutase (SOD) and catalase (CAT) activities, and increasing the liver glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities and reduced glutathione (GSH) level of streptozotocin-induced diabetic rats.

Gongronema latifolium Endl. (Family: Asclepiadaceae; common name: Bush buck)

The origin of the plant is traced to Nigeria in West Africa and the Efiks and Quas in Calabar use *G. latifolium* crude leaf extract in the treatment of malaria, diabetes, hypertension, and as laxative and it is also used as a spice and vegetable (Morebise et al., 2002). Scientific studies have established the hypoglycaemic, hypolipidaemic and antioxidative effects of aqueous and ethanol extracts of *G. latifolium* leaf (Ugochukwu et al., 2003; Ogundipe et al., 2003). Ugochukwu and Babady (2002) reported that *G. latifolium* leaves could exert antidiabetic activities through their antioxidant properties. Morebise et al. (2002) showed that the leaf extract has anti-inflammatory properties. So also, Eleyinmi (2007) investigated the roles of the leaf extract in preservation of food. Some phytochemicals such as B-sistosterol, lupenyl esters, pregnane ester, glycosides, essential oils and saponins are associated with different parts of this herb (Morebise et al., 2002). It is plausible that one or more of these phytochemicals that are found in *G. latifolium* is likely to influence its medicinal activities. Edet et al. (2009) investigated the effect of *G. latifolium* on serum cardiac enzymes in alloxan induced diabetic rat models and normal control rats using graded doses of 80% ethanolic leaf extract of *G. latifolium*. Serum creatine kinase isoenzyme (CKMB) and lactate dehydrogenase (LD) activities increased significantly ($p > 0.001$) in diabetic rats when compared to non-diabetic rats. Serum CK and LD decreased significantly in diabetic and non-diabetic rats treated with *G. latifolium* leaf extract when compared with the control. Moreover, the significantly lowered activities of CK, CKMB and LD at 200 mg/kg body weight and CK and LD at 400 mg/kg body weight scientifically suggest that the leaf extract of *G. latifolium* may have the potential of reducing the factors that produce infarction in the myocardium. This is so because the metabolism of alloxan-induced infarct myocardium may be studied by assessing the level of marker enzyme proteins in the serum. It is interesting to know that as myocardial diseases are rich sources of CKMB, so are skeletal muscular diseases good sources of creatine kinase isoenzyme (Hamm et al., 1992).

Pathological value has been estimated in injured skeletal muscle, therefore the significant reduction in CK enzyme at the dose of 400 mg/kg body weight of *G. latifolium* extract may be due to some physiological effects on muscular activity. This fact may be associated with the efficacy of *G. latifolium* crude leaf extract in the treatment of muscular pains, arthritis and inflammation (Morebise et al., 2002). These data suggest that the effects of *G. latifolium* leaf extract are not dose dependent and hepatotoxic.

DIABETIC INDUCED HYPERLIPIDAEMIA

Hyperlipidaemia is a known complication of DM and coexists with hyperglycaemia and is characterized by increased levels of cholesterol, triglycerides and marked changes in lipoprotein fractions and the control of hyperlipidaemia is a prerequisite for the prevention of diabetic microangiopathy (retinopathy, nephropathy and neuropathy) and macroangiopathy (ischemic heart disease), cerebral vascular disease and arteriosclerosis obliterans in diabetes (Sharma et al., 2013). Oxidative stress in cells and tissues results from the increased generation of reactive oxygen species and/or from diseases in antioxidant defense potentials (Gumiieniczek et al., 2002). Lipid peroxidation of cellular structures, a consequence of free radical activity in turn seemed to play an important role in aging and late complications of diabetes (Ugochukwu and Cobourne, 2003), disrupting natural antioxidant defence systems and altering antioxidant enzyme activities in various tissues like the liver (Rauscher et al., 2001). On the other hand, an increase in circulating lipids may be a reason for increased lipid peroxidation in diabetes.

Currently, there is a renewed and growing interest in the use of plant-based products as drugs or as ‘leads’ in the manufacture of more potent drugs (Ogbonnia et al., 2008). Several secondary plant metabolites have been shown to modify biological processes, which may reduce the risk of chronic diseases in humans (Ugochukwu et al., 2003).

THE USE OF MEDICINAL PLANTS IN THE TREATMENT OF DIABETIC INDUCED HYPERLIPIDAEMIA

Some medicinal plants that have been used in the treatment

of Diabetic Induced Hyperlipidaemia are described.

Helicteres isora **(Family: Sterculiaceae**; **common name: Indian screw tree)**

Helicteres isora is a species of small tree or large shrub found in Asia including India, South china, Malay Peninsula, Java and Saudi Arabia. The bark of *H. isora* has been used in indigenous systems of medicine in India for the treatment of diabetes mellitus since time immemorial. The plant is a shrub or small tree available in forests throughout the Central and Western India. The roots and the bark are expectorant and demulcent and are useful in colic, scabies, gastropathy, diabetes, diarrhoea and dysentery (Kirtikar and Basu, 1995). The fruits are astringent, refrigerant, stomachic, vermifugal, vulnerary and useful in griping of bowels and flatulence in children and possess an antispasmodic effect (Pohocha and Grampurohit, 2001). From the roots, cucurbitacin B and isocucurbitacin B were isolated and reported to possess cytotoxic activity (Bean et al., 1985). The roots have a significant hyperglycaemic effect (Venkatesh et al., 2003). The aqueous extract of the bark showed a significant hypoglycaemic effect (Kumar et al., 2006a), hypolipidaemic activity (Kumar and Murugesan, 2008), lowering effect of hepatic enzymes (Kumar et al., 2006b), and glycoprotein levels (Kumar and Murugesan, 2007) and an antiperoxidative effect (Kumar et al., 2007). The aqueous extract of *H. isora* bark (100 mg, 200 mg/kg body weight) was screened for its antioxidant effect in streptozotocin induced diabetic rats by Kumar et al. (2008). An appreciable decrease in peroxidation products, thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), and hydroperoxides (HP) was observed in the heart tissues of *H. isora* (HI) treated diabetic rats. The decreased activities of key antioxidant enzymes such as SOD, catalase (CAT), GPx, GST and GSH in diabetic rats were brought back to near normal range upon HI treatment. The results suggest that the effectiveness of the drugs depends, probably, on the accumulative effect of active principles (Peungvicha et al., 1998). From the roots and barks of HI, betulic acid, daucosterol, sitosterol, isorin were isolated (Qu et al., 1991). Furthermore, the present study of Kumar et al. (2008) therefore provides some useful insight into the cardiac antioxidant and antiperoxidative potency of bark of HI in STZ induced diabetes.

Eleucine coracana **(Family: Poaceae; common name: Black finger millet)**

Eleucine coracana is an annual widely grown cereal in the arid areas of Africa and Asia. The millet seed coat contains several phenolic compounds like phenolics, flavonoids, polymeric tannins and anthocyanins, some of which are effective inhibitors of pancreatic amylase and intestinal α-glucosidase (Chethan and Malleshi, 2007). It is also a rich source of phytates and minerals (Shobana et al., 2006). Traditionally, finger millet food preparations are known for their higher sustaining power, lower glycaemic response and higher satiety scores compared with other cereal foods which are usually recommended for diabetic patients. Dietary polyphenols and phytates are known for their ability to reduce carbohydrate digestibility and thereby regulate postprandial glycaemic response (Thompson et al., 1987). Moreover, polyphenols are known to inhibit glucose absorption and prevent advanced glycation end product (AGE) formation (Scalbert et al., 2005).

Okoyomoh et al. (2013) evaluated the antioxidant and antidiabetic properties of seed coat matter (SCM) of grains of black finger millet on 20 and 40% in streptozotocin induced diabetic rats. *E. coracana* exhibited significant antidiabetic activity resulting to a 45% reduction in the diabetic experimental group treated with 40% SCM. Apart from being a rich source of dietary fibre, phytates and minerals, the millet seed coat is a reserve of many health-beneficial phenolic compounds (Shobana et al., 2006; Chethan and Malleshi, 2007). It has been reported that polyphenols reduce fasting hyperglycaemia and attenuate the postprandial blood glucose response in rats (Scalbert et al., 2005). Feeding the experimental rats with various concentration of the SCM exhibited significant protective effect by lowering the serum levels of ALT, AST and ALP. Catalase (CAT) and superoxide dismutase (SOD) activities were increased while the concentration of thiobarbituric acid reactive substances (TBARS) was significantly lowered. Phytate is known to have amylase-inhibitory properties (Knuckles and Betschart, 1987) and a regulatory role in insulin secretion from pancreatic beta cells.

Earlier reports have shown that finger millet phenolics are non-competitive inhibitors of intestinal α-glucosidase and pancreatic amylase (Shobana et al., 2009). As these inhibitors are proven modulators of postprandial glycaemia, they play a significant role in the management of diabetic complications. Phenolic compounds are also known to enhance insulin activity (Anderson and Polansky, 2002), regulate intestinal glucose transporter (GLUT2) (Shimizu et al., 2000), increase muscle glucose uptake and reduce hepatic gluconeogenesis (Liu et al., 1999). Hence, the phytate of the SCM may have complemented the positive role of polyphenols towards regulation of postprandial glycaemia and ameliorating complications associated with diabetes via impeding glucose absorption in the small intestine. There was significant reduction in liver enzyme activity; this could be attributed to the polyphenolic content of the seed coat

matter. Histopathological observations also revealed that SCM of *E. coracana* offered protection to the animals against pancreatic, kidney and liver STZ induced damages. The result indicates that the various concentrations of *Eleusine coracana* grains possess antioxidant and antidiabetic potentials in STZ induced diabetic rats (Okoyomoh et al., 2013).

Pterocarpus santalinus (Family: Fabaceae; common name: Red sandal wood)

Pterocarpus santalinus Linn (Fabaceae) (PS), commonly known as "Red sanders", is a small to medium sized deciduous tree, 7.5 m high, with an extremely hard, dark purple heart-wood with a bitter flavor. In the traditional system of medicine, the decoction prepared from the heartwood is attributed to various medicinal properties. It has been used as a cooling agent, antipyretic, anti-inflammatory, antihelmintic, tonic, hemorrhage, dysentery, aphrodisiac, diaphoretic as well as to induce vomiting, to treat eye diseases, mental aberrations and ulcers (Kiritikar and Basu, 1987). The wood in combination with other drugs is also prescribed for snake-bites and scorpion-stings (Warrier et al., 1995). Decoction of the heartwood has been reported as a central nervous system (CNS) depressant and also shown to have anti-inflammatory activity for induced hind paw edema in rats when prepared in formalin (3%). Himoliv, a polyherbal Ayurvedic formulation containing PS as one of the ingredients, has been reported to possess hepatoprotective activity (Bhattacharya et al., 2003).

Heartwood contains pterocarpol, santalin A, B, pterocarptriol, ispterocarpolone, pterocarpo-diolones with *β*-eudeslol and cryptomeridol (Yoganarasimham, 2000). In addition, Aurone glycosides viz., 6-OH-1-Methyl-3', 4', 5'-trimethoxyaurone-4-*O*-rhamnoside and 6, 4'-dihydroxyaurone-4-*O*neohesperidoside, and isoflavone glycoside 4', 5-dihydroxy 7-*O*-methyl isoflavone 3'-*O*-beta-*D*-glucoside are also present (Krishnaveni and Rao, 2000). In addition, the bark extract has a blood glucose level lowering effect in experimental animals (Varma et al., 1991). Methanol and aqueous extracts of heartwood of PS have shown anti-hepatotoxicity in CCl_4-induced hepatoxicity (Rane and Gramarc, 1998). Halim et al. (2011) reported that treatment with *P. santalinus* caused significant lowering of blood sugar and improvement in glucose tolerance tests and a decrease HbA1c on regular long term control over blood glucose levels was observed. The antioxidant properties of the red sandal wood extract was also evident, as it caused a reduction in MDA in the brain, liver and muscle tissues. The extract also caused a decrease in the formation of lipid peroxidase, estimated by TBARS and increased antioxidants SOD, CAT, glutathione peroxidase and glutathione transfers in erythrocytes. Serum creatinine and urine albumin showed decreased levels after treatment with *P. santalinus* and thereafter returned to control values. The kidneys examined histologically for diabetic nephropathy showed regression following treatment. Furthermore, sixteen weeks combination therapy also resulted in decreases in LDL-C/HDL-C, TC, TG and an increase in HDL-C of treated diabetic rats. The use of the aqueous extract of *P. santalinus* caused improvements in glycaemia, lipid peroxidation and brain, liver and heart masses due to its antioxidant properties (Halim et al., 2011).

Physalis angulata (Linn) (Family: Solanaceae; common name: Ground cherry)

Physalis angulata, a branched erect annual plant, is widely distributed throughout tropical and subtropical regions of the world and mostly abundant as a weed in gardens, waste lands and pastures, plantations, along roads, in forest along creeks near sea levels and even in cultivated fields (Smith, 1991; Januario et al., 2002). It is a medicinal plant employed in herbal medicine around the world for the treatment of various diseases such as hepatitis, asthma, malaria, dermatitis and rheumatism (Soares et al., 2003). The plant was reported to possess central nervous system depressant effects (Oladele et al., 2013). Some compounds such as physalin A, B, D and F, and glycosides (myricetin-3-o-neohesperidoside) have been isolated from the organic fractions of the plant. Oladele et al. (2013) studied the antidiabetic potentials of the ethanolic root extract of the plant using alloxan induced diabetes mellitus in rats. The 400 and 800 mg/kg of the extract significantly ($p < 0.05$) reduced the blood glucose, cholesterol, triglycerides and low density lipoproteins, while the high density lipoproteins significantly ($p < 0.05$) increased. This may suggest that the mechanisms of actions of ethanolic root extract of *P. angulata* could be by beta cell regeneration in addition to the possibility of stimulation of glucose utilization in the liver and peripheral tissues through the keyenzymes participating in glucose metabolism. It was therefore concluded that the ethanolic root extract of the plant possesses antidiabetic properties as well as anti hyperlipidaemic effects.

Bauhinia forficata Link. (Family: Caesalpinaceae; common name: Orchid Tree)

Orchid tree is a perennial shrub that can be found in the rain forest. It is frequently used as anti-diabetic herbal medicine. It is also used as diuretic for kidney and urinary

disorders (including polyuria, cystitis and kidney stones), as a blood cleanser, to build blood cells and for high cholesterol. Oral administration of kaempferilrin, a major flavonoid compound of the n-butanol fraction from *B. forficata* leaves leads to a significant hypoglycemic effect in normal and in alloxan-induced diabetic rats. In normal rats, kaempferitrin lowers blood glucose only with the higher dose of 200 mg/kg at 1 h after treatment and also shows antioxidant properties (de Sousa et al., 2004). Administration of aqueous, ethanolic and hexanic extracts daily for 7 days at doses of 200 and 400 mg/kg, to the alloxan-diabetic rats, shows significant reductions in plasma glucose, triglycerides, total cholesterol and HDL-cholesterol after treatment with the extracts and glibenclamide (standard drug) as compared to the diabetic controls (Lino et al., 2004).

Vitex doniana (family; Verbanaceae; Common Name: Vitex)

Vitex doniana is a perennial shrub widely distributed in tropical West Africa, and some East African countries including Uganda, Kenya and Tanzania (Yakubu et al., 2012). It is found in the middle belt of Nigeria particularly Kogi, Benue, and parts of the savannah regions of Kaduna, Sokoto and Kano states. It is variously called *dinya* (Hausa), *dinchi* (Gbagyi), *ucha koro* (Igbo), *oriri* (Yoruba), *ejiji* (Igala) and *olih* (Etsako) in Nigeria (Yakubu et al., 2012). *V. doniana* is employed in the treatment of a variety of diseases. Hot aqueous extracts of the leaves are used in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhea, dysentery and diabetes indicating that the plant's leaves may possess antidiabetic properties among others and in North-Central and eastern parts of Nigeria, the young leaves are used as vegetables or sauces and porridge for meals, especially for diabetic patients (Yakubu et al., 2012). Yakubu et al. (2012) investigated the effect of aqueous leaf extract of *V. doniana* on oxidative stress and lipid peroxidation in streptozotocin-induced diabetic and non diabetic rats.

The results indicated that the concentrations of TBARS, AST, ALT, ALP and bilirubin were significantly ($p < 0.05$) increased while the activities of CAT and SOD were reduced in the diabetic animals. The extract significantly increased CAT and SOD activity and reduced TBARS, ALT, AST, ALP and bilirubin concentrations ($p < 0.05$). These reductions in TBARS, ALT, AST, ALP and bilirubin concentrations could lead to a decrease in oxidative stress and hence a reductions in the rate of progression of diabetic complications in the liver. The study concluded that the extract reversed diabetes - induced oxidative changes in the hepatocytes, thus suggesting its use for the management of diabetic complications.

Albizia lebbeck Benth (Family: Fabaceae; common name: Lebbek, woman's tongue tree, flea tree, fry wood and koko)

Albizia lebbeck Benth. is a deciduous tree with compound leaves and flat oblong fruits. It is distributed throughout India from the plains upto 900 m in the Himalayas. The bark and flowers of *A. lebbeck* were used to treat arthritis according to the Siddha system of Medicine (Jain, 1991). Hypoglycaemic and/or anti-hyperglycaemic activities have been recorded with numerous plants, many of which are used as traditional herbal treatments of diabetes. *A. lebbeck* Benth. stem bark have been used in traditional medicine along with some preliminary reports on its hypoglycaemic action. Ahmed et al. (2014) evaluated the antidiabetic and antioxidant activities of methanolic extract of stem bark of *A. lebbeck* in streptozotocin induced diabetic rats. Streptozotocin induced diabetic rats depicted the increased blood glucose levels, TC, TG, LDL-c, diminished HDL-c level and perturb level of antioxidant markers. Oral administration of methanolic extract of stem bark of *A. lebbeck* at a concentration of 100, 200, 300 and 400 mg/kg b.w daily for 30 days results in a momentous decrease in fasting blood glucose, glycated haemoglobin and enhancement of plasma insulin level as compared with STZ induced diabetic rats. Furthermore, it significantly ($p < 0.05$) decreased the level of TC, TG, and LDL-c, VLDL-c, while it increased the level of HDL-c to a significant ($p < 0.05$) level. The treatment also resulted in a marked increase in reduced glutathione, glutathione peroxidase, catalase and superoxide dismutase and diminished level of lipid peroxidation in liver and kidney of STZ induced diabetic rats.

Histopathological studies suggest the diminution in the pancreatic, liver and cardiac muscle damage. Their research exertion clearly indicates the considerable antihyperglycemic, antihyperlipidaemic, antioxidant and pancreas/renal/hepatic/cardiac protective action of methanolic extract of stem bark of *A. lebbeck*.

Caesalpinia bonducella (L) Roxb. (Family: Caesalpinaceae; common name: Fever nut)

Caesalpinia bonducella F. (Leguminosae) is a medicinal plant, widely distributed throughout India and the tropical regions of the world. Its seed kernels are used in the management of diabetes mellitus, in the folklore medicine of Andaman and Nicobar as well as the Caribbean Islands. It has been reported that seeds of the plant possess antidiarrhoeal, antiviral, antibacterial, anti-microbial, antifungal, antidiabetic, antitumor, antipyretic and analgesic, antifilarial, anxiolytic, anti- inflammatory,

antioxidant, immunomodulatory, adaptogenic, anticonvulsant, antispasmodic, nootropic, antifeedant, antiamoebic, antioestrogenic, diuretic, insecticidal, as well as trypsin and chymotrypsin inhibitor properties (Nazeerullah et al., 2012; Moon et al., 2010; Kshirsagar, 2011; Emmanuel and Swaran, 2006). Phytochemical analysis of *C. bonducella* seeds has revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids. Phytochemical studies on ethanolic extracts of the bark of *C. bonducella* yielded two new homoisoflavonoids along with five known natural products and all of these compounds exhibited different levels of GST inhibitory and antifungal potentials (Ata et al., 2009). The oral administration of the extracts (300 mg/kg) produces significant antihyperglycemic action as well as lowers the bilirubin levels significantly.

The action of the extracts on diabetes induced hyperlipidaemia significantly lowers the elevated cholesterol as well as LDL level. The antihyperglycemic action of the extracts may be due to the blocking of glucose absorption. The drug has the potential to act as antidiabetic as well as antihyperlipidaemic (Kannur et al., 2006).

Aloe arborescens (Family: Liliaceae; common name: Aloe vera)

Aloe arborescens is a cactus like plant that grows readily in hot, dry climates and currently, because of demand, is cultivated in large quantities (Grieve, 1975). There are some preliminary studies to suggest that oral administration of *Aloe vera* might be effective in reducing blood glucose levels in diabetic patients and in lowering blood lipid levels in hyperlipidaemia (Rajasekaran et al., 2004). Sharma et al. (2013) examined the potential antihyperlipidaemic and antihyperlipidaemia efficacy of the aqueous extract from *A. vera* leaf gel in alloxan-induced diabetic mice. Various biochemical parameters, including lipid profile and serum glucose were decreased as well as HDL-C increased. It may be followed that the extract acts as an antioxidant blocking the formation of the reactive oxygen species; mechanism of which remains to be elucidated. This result however demonstrates the antidiabetic and antihyperlipidaemic activities of *A. vera* leaf extract in diabetic mice.

Nigella sativa L (Family: Ranunculaceae; common name: Black cumin)

Nigella sativa Linn. (NS) is a small medicinal herb whose different parts have been reported as therapeutic agents in traditional system of medicines. The seeds of NS are considered carminative, stimulant, diuretic and used in the treatment of mild cases of puerperal fever; they are externally applied for eruptions of skin. Oral administration of ethanol extract of *N. sativa* seeds (300 mg/kg body weight/day) to streptozotocin induced diabetic rats for 30 days significantly reduces the elevated levels of blood glucose, lipids, plasma insulin and improved altered levels of lipid peroxidation products (TBARS and hydroperoxides) and antioxidant enzymes like catalase, superoxide dismutase, reduced glutathione and glutathione peroxidase in liver and kidney. The results confirm the antidiabetic activity of *N. sativa* seeds extract (Kaleem et al., 2006).

Ficus exasperata (Family: Moraceae; common name: Sandpaper leaf tree)

The sandpaper leaf tree is a terrestrial afro-tropical shrub or small trees. In traditional medicine, different parts of this plant (fruit, leaf, sap, bark, and root) are considered medicinally important. In Africa, Yemen and India, various parts of the plant are used as analgesic, antiarthritic, diuretic vermifuges, febrifuge, abortifacient, ecbolic, wound healing, animal fodder and also in general debility, malnutrition, parasitic infection (cutaneous, subcutaneous), leprosy, ophthalmic and oral infections, nasopharyngeal afflictions, arthritis, rheumatism, gout, edema, kidney disorders, diarrhea, dysentery, hemorrhoids and venereal diseases.

The effects of treatment with aqueous extract of *Ficus exasperata* on the pathophysiology and histopathology in alloxan-induced diabetic rats was studied by Adeyi et al. (2012). Hyperglycaemia was recorded in all induced rats after alloxan induction, while treatment with the different concentrations of the plant extract reversed hyperglycaemia within four days. Values of packed cell volume (PCV), haemoglobin concentration (Hb) and RBC were higher in rats treated with the extract than in rats treated with glibenclamide, while ionoregulatory distruptions observed in the diabetic groups reduced significantly ($p < 0.05$) in rats treated with the extract. Lipid profile parameters were higher in rats treated with glibenclamide compared to groups treated with the extract.

Treatment with the plant extract ameliorated the various degenerations observed in the pancreas, liver and kidney in contrast to untreated group and group treated with glibenclamide. Results from this study demonstrated the ameliorative effects of aqueous leave extract of *F. exasperata* on the pathophysiological and histopathological complications of diabetes mellitus.

Ocimum sanctum L. (Family: Lamiaceae; common name: Holy Basil)

It is a tropical annual herb grown all over India and use

for household remediation (Mukherjee et al., 2006). Since ancient times, this plant has been known for its medicinal properties. The aqueous extract of the leaves show significant reduction in blood sugar level in both normal and alloxan induced diabetic rats (Vats et al., 2002). Significant reduction in fasting blood glucose, uronic acid, total amino acid, total cholesterol, triglyceride and total lipid indicate the hypoglycemic and hypolipidaemic effects of tulsi in diabetic rats (Rai et al., 1997). *Ocimum sanctum* leaf powder produced potent hypoglycemic and hypolipidaemic effect in normal and diabetic rats (Ravi et al., 2004).

Pterocarpus marsupium Roxb. (Family: Papilionaceae; common name: Indian kino tree)

Pterocarpus marsupium, also known as Vijayasar or the Indian Kino Tree, is a medium to large tree that can grow up to 30 m tall. It is widely used in 'Ayurveda' as 'Rasayana' for management of various metabolic disorders. An aqueous extract of *P. marsupium* wood, at an oral dose of 250 mg/kg, shows statistically significant hypoglycemic activity (Mukhtar et al., 2005). Marsupin, pterosupin and liquiritigenin obtained from this plant show antihyperlipidaemic activity (Jahromi and Ray, 1993). (-)Epicatechin, its active principle, has been found to be insulinogenic, enhancing insulin release and conversion of proinsulin to insulin *in vitro*. Like insulin, (-)epicatechin stimulates oxygen uptake in fat cells and tissue slices of various organs, increases glycogen content of rat diaphragm in a dose dependent manner (Ahmad et al., 1989).

Trigonella foenum graecum L. (Family: Papilionaceae; common name: Fenugreek/Menthi)

Trigonella foenum -graecum (Linn.) belonging to the family Papilionaceae commonly known as Fenugreek is an aromatic, 30 to 60 cm tall, annual herb, cultivated throughout the country (Yadav et al., 2014). Menthi is used both as a herb (the leaves) and as a spice (the seed) and cultivated worldwide as a semi-arid crop. Oral administration of 2 and 8 g/kg of plant extract produces dose dependent decrease in the blood glucose levels in both normal as well as diabetic rats (Khosla et al., 1995). Administration of fenugreek seeds improves glucose metabolism and normalizes creatinine kinase activity in heart, skeletal muscle and liver of diabetic rats. It also reduces hepatic and renal glucose-6-phosphalase and fructose -1,6-biphosphatase activity (Gupta et al., 1999). Compounds extracted from the plant have shown cardiotonic, hypoglycaemic, diuretic, antiphlogistic, hypotensive activity and hypocholesterolemic properties. Its major free amino acid 4-hydroxyisoleucine stimulates insulin secretion from perfused pancreas *in vitro* (Al-Habbori and Raman, 1998). The galactomannan-rich soluble fiber fraction of fenugreek may be responsible for the antidiabetic activity of the seeds (Yadav et al., 2014). Insulinotrophic and antidiabetic properties also have been associated with the amino acid 4-hydroxyisoleucine that occurs in fenugreek at a concentration of about 0.55%. *In vitro* studies have indicated that this amino acid causes direct pancreatic betacell stimulation. Delayed gastric emptying and inhibition of glucose transport also have been postulated as possible mechanisms (Yadav et al., 2014).

DIABETES INDUCED ERECTILE DYSFUNCTION (DIED)

Impotence or erectile dysfunction is defined as inability to achieve and/or maintain an erection sufficient to permit satisfactory sexual intercourse (Barve, 2013). Erectile dysfunction often is seen as a result of diseases such as diabetes, kidney disease, chronic alcoholism, multiple sclerosis, atherosclerosis, vascular diseases and neurological diseases. Amongst these disorders, diabetes and associated oxidative stress are major contributors for impotency in males. An association with diabetes and erectile dysfunction has been documented since 1798 (McCulloch et al., 1980). It has been reported that 35 to 50 percent of men with diabetes experience erectile dysfunction (National Institutes of Health (NIH) Erectile Dysfunction, 2004). It is usually present within 10 years of diagnosis. The presence of diabetes mellitus not only increases the risk for ED but also other aspects of sexual dysfunction which include sexual drive, ejaculatory function and sexual satisfaction (Burke et al., 2007). The pathophysiology for DIED is multifactorial and it is associated with hyperglycaemia and protein glycosylation thus leading to the production of AGEs (Wolff and Dean, 1987)

These might contribute to DIED either by generating free radicals which in turn quench nitric oxide or damage the potassium channels, both of which are required for the cavernosal smooth muscle relaxation (Giuseppe et al., 2006). There is an elevation of endothelins, which are potent vasoconstrictors in the penis, which inhibit the relaxation (Mills et al., 2001). RhoA/Rho kinase is implicated in decreased production of NO in the penis, which in turn might be responsible for ED (Rees et al., 2002). DIED might also be a consequence of neuropathic damage (Costabile, 2003). Impairment of cGMP dependant protein kinase 1 (PKG-1) plays an important role in DIED (Chang et al., 2004).

Although there are several drugs available in the market, there are limitations in their use either due to high

cost or side effects like hypoglycaemia, weight gain, gastrointestinal disturbances, liver toxicity etc (Dey et al., 2002). In search of first line treatment with better safety and efficacy, research efforts have to be made to find a complete treatment of DIED. The ideal drug to combat DIED is one which involves the NO/cGMP pathway, but a combination of drugs affecting multiple peripheral intracellular targets could also be an option available for treatment.

THE USE OF MEDICINAL PLANTS IN THE TREATMENT OF DIABETES INDUCED ERECTILE DYSFUNCTION

Medicinal plants are currently being researched for the treatment of diabetes and its complications. The World Health Organizatiobn (WHO) has listed 21,000 plants which are used for medicinal purposes around the world. Of these, 2500 species are found in India (Seth and Sharma, 2004). Some of the herbs used in traditional systems of medicine in India and world over are reviewed in detail for their aphrodisiac and antidiabetic effect. These herbs could be promising candidates for exploring their potential in the treatment of DIED, due to their combined effects on erectile dysfunction and diabetes.

Chlorophytum borivilianum (Family: Asparagaceae; common name: Safed musli)

Safed musli (*Chlorophytum borivilianum*) is a herb, and belongs to family Liliaceae. It was originally grown in thick forests of India (Singh et al., 2012). About 300 species are distributed throughout the tropical and subtropical parts of the world. Tropical and subtropical zones of Africa are the probable centres of origin of the genus. The tubers have been traditionally used for various therapeutic applications. It is used as an aphrodisiac, treatment of diabetes and arthritis, curative for prenatal and postnatal problems etc. The roots contain two major constituents-saponins and mucilage. The roots (tubers) are considered rich in alkaloids, vitamins, minerals, proteins, carbohydrates, saponins, polysaccharides and steroids. It has various therapeutic values as total rejuvenator, antioxidant and Immunomodulator (Singh et al., 2012). It has been found that the fructo-oligopolysaccharide fraction is effective in treatment of streptozotocin induced diabetes (Narsimhan et al., 2006). Furthermore, it is found that the same fraction is also effective in the treatment of sexual dysfunction in hyperglycemic male rats. The probable mechanism behind this effect is improved steroidogenesis and rejuvenation of the entire system that helps in restoring the failing sexual function in diabetes (Thakur et al., 2009).

Dioscorea bulbifera (Family: Dioscoreaceae; common name: Shoebutton air potato, air yam, bitter yam)

Air potato, *Dioscorea bulbifera*, is an invasive plant not native to Florida but whose present-day distribution includes most of the state (Hammer, 1998). It is a vigorously twining, long-stemmed herbaceous vine which may arise from an underground tuber, although often tubers are inconspicuous or absent. The stems are round to slightly angled in cross section and they twine counter-clockwise. Conspicuous aerial tubers (called bulbils) are pale, round to globose in shape, up to 13 cm wide and are formed in leaf axils. It is these bulbils that give *D. bulbifera* the common name "air potato' (Langeland, 2001). *D. bulbifera* containing steroidal saponin based on diosgenin is also believed to act on the seminiferous tubules presumably by exerting a testosterone like effect (Park et al., 2006). Extract prepared from the bulbs of *Dioscorea* is found to inhibit alpha-amlyase and alpha-glucosidase, thus helping to manage post prandial hyperglycaemia (Ghosh et al., 2012).

CONCLUSION

Diabetes mellitus is a chronic metabolic disorder of impaired carbohydrates, fat and protein metabolism. Limiting diabetes mellitus without any side effects is a challenge still to the medical system. In recent years, herbs have become a subject of interest because of their beneficial effects on human health. Several plant extracts have been examined for their antidiabetic properties in an attempt to recognize alternative treatment strategies that pose less of a hazard for diabetics. The present review article therefore explored the roles of herbs in treatment of diabetes and diabetic complications such as neuropathy, nephropathy, gastropathy, retinopathy, cardiovascular diseases, hyperlipidaemia and erectile dysfunction.

Conflict of interests

The author(s) have not declared any conflict of interests.

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Short Communication

Lipid profile status of streptozotocin induced diabetic rats treated with ethanolic leaf extract of *Solenostemon monostachyus*

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Lipid profile status of streptozotocin induced diabetic rats treated with ethanolic leaf extract of *Solenostemon monostachyus* was assessed. A total of 24 rats were used for the experiments and were divided into four groups (that is, Diabetic Control (DC), Normal Control (NC), Insulin Treated (INS) and *S. monostachyus* (SM) treated groups), with 6 rats each. The extract, 250 mg/kg body weight, was administered twice daily for 21 days. The serum glucose level in mg/dl was 70.00±10.50 for SM treated groups, 256.00±15.00 for DC. There was significant decrease at p>0.05 in blood glucose of rats administered with the extract. The lipid profile values for SM were triacylglycerol (TG, 58.00±2.00), total cholesterol (TC, 35.00±1.00), high density lipoprotein cholesterol (HDL-c, 35.00±1.00), very low density lipoprotein cholesterol (VLDL-c, 16.90±0.30), low density lipoprotein cholesterol (LDL-c, 14.70±0.53) and for DC were TG (155.47±2.76), TC (137.88±5.91), HDL-c (31.94±2.93), VLDL-c (27.54±1.15), LDL-c (95.99±4.72). The result for the lipid profile of SM treated groups showed significant decrease (P>0.05), when compared with DC groups. Based on the results obtained from this study, it may be concluded that the ethanolic leaf extract of SM has hypoglycaemic properties and was able to alleviate elevations in lipid profile and oxidative stress induced by streptozotocin in Wistar albino rats.

Key words: Solenostemon monostachyus, hypoglycaemic, oxidative stress.

INTRODUCTION

Origin of medicinal plant

According to medicinal history, Hippocratus was the first Greek to regard medicine as a science and was also called father of medicine. His “Material Medica” consisted essentially of herbal recipes, some of the medicinal plants described by Hippocratus, included opium, mint, etc., various preparations of aromatic roots and flowers in treating many ailments (Farnsworth and Morris, 1976). Galen in his middle ages was considered to be the most distinguished physician of antiquity after Hippocratus. He treated disease essentially by the use of herbs and those who followed his method eventually developed the sect known as “Electics” who employed herbs as well as mineral substances in treating the sick.

According to Treasure (2002), allopathic as well as

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homeopathic systems of medicine today are based on the doctrines expatiated by Galen. Historically, plants provide a source of inspiration for novel drug compounds. Medicinal plants have made large contributions to human health and wellbeing. Their role is in two fold in the development of new drugs: (1) they may become the base for the development of new drugs; (2) phyto-medicinal for the treatment of disease.

There are numerous examples of plant derived drugs. Some selected examples are isoquinolines, alkaloid, emetine obtained from the underground part of cephaclisipecacuanta and related species. Plant parts have been used for many years as amobicidal drugs as well as for the treatment of abscesses. Another important drug of plant origin with a long history of use is quinine, which is an alkaloid that occurs naturally in the bark of Ginchona tree. Apart from its continued usefulness in the treatment of malaria, it can also be used to relieve nocturnal leg cramps; Nelson (2002) found that higher plants have made important contribution in areas, such as cancer therapies.

Early examples include the anti-leukaemia alkaloids vinblatine and Vincristine which are both obtained from the Madagascan periwinkle plant. *Solenostemon monostachyus* could serve as a good supplement because of its hydrogen peroxide scavenging potential. Erythrocytes are the most abundant cells in vertebrates. Their unique morphology and physiological nature are exploited in drug delivery and targeting. Because of the preponderance of polyunsaturated fatty acids in the erythrocyte membranes, they are highly susceptible to oxidative damage whose consequences are lipid per-oxidation and haemolysis. This has been a mechanism for erythrocyte cell injury and death (Miki et al., 1987).

Erythrocyte haemolysis can be caused by some haemoglobinopathies, oxidative drugs and redox active metals (Ko et al., 1997). *S. monostachyus* is a prime candidate that could reverse the oxidative damages. The following compounds have been isolated from the leaves of *S. monostachyus*. They are the terpenoids β- pinene, oct- 1- en – 3-ol, β- caryophyllene, octan-3-ol, and E, E-α-farnesene (Eyele Mve et al., 2006). Dietary polyphenols, carotenoids and terpenes have also exhibited significant medicinal potentials (Wang et al., 2008).

Diabetes mellitus

Diabetes is a group of metabolic diseases in which a person has high blood sugar, because the pancreas does not produce enough insulin (WHO, 2006). This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).

Types of diabetes

There are three main types of diabetes mellitus (DM).

(1) Type 1 DM results from the body's failure to produce insulin, and presently requires the person to inject insulin or wear an insulin pump. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes".
(2) Type 2 DM results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. This form was previously referred to as non-insulin-dependent diabetes mellitus (NIDDM) or "adult-onset diabetes".
(3) The third main form, gestational diabetes occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level. It may precede development of type 2 DM.

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes. All forms of diabetes have been treatable since insulin became available in 1921, and type 2 diabetes may be controlled with medications. Both types 1 and 2 are chronic conditions that cannot be cured. Pancreas transplants have been tried with limited success in type 1 DM; gastric bypass surgery has been successful in many with morbid obesity and type 2 DM. Gestational diabetes usually resolves after delivery. Diabetes without proper treatments can cause many complications. Acute complications include hypoglycaemia, diabetic ketoacidosis, or nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease (CVD), chronic renal failure, and diabetic retinopathy (retinal damage). Adequate treatment of diabetes is thus important, as well as blood pressure control and lifestyle factors such as smoking cessation and maintaining a healthy body weight.

MATERIALS AND METHODS

Identification and preparation of plant

Fresh leaves of *S. monostachyus* were collected from Botanical garden at the University of Calabar, Calabar, Nigeria. The sample of the plant specimen was identified and authenticated by a Botanist from the botanical garden, and the voucher specimen was deposited in the herbarium of the same university. The leaves were sorted to eliminate any dead matter and other unwanted particles. The leaves were air-dried for 2 weeks and the leaves were blended with a manual hand blender and 150 g of the plant was weighed and soaked in 700 ml of ethanol. The mixture was then placed in a water bath at about 60 to 80°C for 10 min for thorough extraction of the plant active components and allowed to cool. The extract was then filtered with a chess material and later a Whatman no. 1 filter paper to obtain a homogenous filtrate. The filtrate was then concentrated *in vacuo* at low temperature of 37 to 40°C. The concentrate was then allowed open in a water bath for complete dryness. The extract was refrigerated at 2 to 5°C until when used. Appropriate concentration of the extract was subsequently made by dilution with distilled water into 250 mg/kg body weight and

Table 1. Animal groupings and treatment schedule/protocol.

Group	Number of rats	Treatment/Vehicle
Normal control (NC)	6	50% DMSO and distilled water
Diabetic control (DC)	6	50% DMSO and distilled water
Insulin treated group (INS)	6	Insulin (5 unit/kg body weight)
Solenostemon monostachyus treated group (SM)	6	Extract (250mg/kg body weight)

Table 2. Effect of treatment on glucose levels in blood and serum.

Group/Treatment	Initial FBG (mg/dl)	Final FBG (mg/dl)	Serum glucose (mg/dl)
NC	74.83±8.92	90.50±6.20	57.14±8.12
DC	539.33±14.13*	323.00±8.58*	256.00±15.00*
INS	433.33±32.24^{*a}	82.33±2.47^{a}	54.93±2.32^{a}
SM	297.25±57.69^{*a}	191.33±4.94^{a}	70.00±10.50^{a}

Values are expressed as mean ± SEM. *Significantly different from NC at $p<0.05$. ^{a}Significantly different from DC at $p<0.05$. ^{b}Significantly different from INS at $p<0.05$. ^{c}Significantly different from SM at $p<0.05$.

administered to the animals.

Handling and treatment of animals

A total of 24 adult male albino rats weighing between 150 and 250 g obtained from the disease free stock of the animal house, Biochemistry Department, College of Medical Sciences University of Calabar, Calabar, Nigeria, were used for the study. The rats were divided into four groups with six rats each, as follows: Group A (normal control group receiving distilled water as placebo), Group B (diabetic control group receiving distilled water as placebo), Group C (insulin group receiving 5 unit/kg body weight of insulin) and Group D (diabetic test group I received oral dose of *S. monostachyus* leaves extract juice). Table 1

The dose employed during administration was based on the predetermined LD_{50} values obtained from preliminary studies.

Induction of experimental diabetes

Prior to diabetes induction, the rats were subjected to 12 h fast, and then diabetes was induced by intraperitoneal injection of 40 mg/kg body weight with streptozotocin (STZ) (sigma St. Louis, Mo, U.S.A) using sodium citrate buffer (0.5M) reconstituted in dimethyl sulfoxide (DMSO). The normal control animals received DMSO only, three days after STZ treatment, diabetes was confirmed in STZ treated rats with a fasting blood sugar (FBS) concentration ≥ 200 mg/dl. This was estimated using One Touch Glucometer (Lifescan, Inc. 1996 Milpas, California, U.S.A) with blood obtained from the tail vein of the rats.

The rats were acclimatized in the experimental animal house for one week before the commencement of the experiment. The animals, housed in stainless steel cages under standard conditions (ambient temperature 28.0±2.0°C and humidity 46%, with a 12 h light/dark cycle), were fed with the normal rat pellets. All the rats in both test and control groups were allowed free access to food and water *ad libitum*, throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily. The animals in test group III received insulin 5 unit/kg body weight and group IV received oral daily doses of aqueous extract of *S. monostachyus* leaves, respectively, using orogastric tubes and syringes. This lasted for a period of 21 days and the experiments were conducted between the hours of 7.00 am and 7.00 pm daily. Rats in the control groups I were administered, by oral gavage, with 5 ml of distilled water (placebo). All the animal experiments were carried out in accordance with the guidelines of the Institution's Animal Ethical Committee (Table 2).

Collection and analysis of blood

All the animals were anaesthetized with chloroform vapour, twenty-four (24 h) after last day of extract administration, and dissected for blood collection. Blood samples were collected by cardiac puncture into a set of plain and fluoride oxalate sample bottles. The determination of LD_{50}/Acute toxicity tests was done orally using albino mice.

Biochemical tests were carried out, which include: liver enzymes-alanine aminotransferase and aspartate aminotransferase according to the method described by Reithman and Frankel (1957). Serum alkaline phosphatase was done using Randox test kits. Lipoprotein profile-total cholesterol was done using HDL-C, TG, serum electrolytes, urea and creatinine.

Statistical analyses

The results obtained from this study were analyzed by one-way analysis of variance (ANOVA), followed by Student's t-test to evaluate the significance of the difference between the mean value of the measured parameters in the respective test and control groups using SPSS windows. A significant change was considered acceptable at $P>0.05$.

Changes in blood glucose of diabetic and non-diabetic test animals

Daily changes in blood glucose were monitored following daily

Table 3. Effect of treatment on serum lipid profile.

Group	TG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)
NC	68.67±2.88	105.66±12.71	37.57±4.60	21.14±2.55	39.98±5.54
DC	155.47±2.76*	137.88±5.91*	31.94±2.93	27.54±1.15*	95.99±4.72*
INS	66.91±2.20[a]	119.90±12.08[a]	36.08±1.22	23.98±2.42	46.84±1.48[a]
SM	58.00±2.00[a]	24.00±0.58[a*]	35.00±1.00	16.90±0.30[a]	14.70±0.53[a]

Values are expressed as mean ± SEM. *Significantly different from NC at p<0.05. [a]Significantly different from DC at p<0.05. [b]Significantly different from INS at p<0.05. [c]Significantly different from SM at p<0.05.

treatment with extract and insulin in diabetic and non-diabetic rats. The blood glucose levels ranged between 60 and 108 mg/dl in the normal control rats (NC) that were not given any extract. Fasting blood glucose of other treatment groups varied differently. The treatment groups tended toward lowering the blood glucose levels when compared with the diabetic control (DC), but the *S. monostachyus* (SM) group was closely related to the insulin treated group. The diabetic control group range was between 601 and 295 mg/dl. Differences in DC showed sustained elevations in blood glucose level of untreated diabetic rats.

Effect of treatment on serum lipid profile

Changes in serum lipids concentration following treatment, in this investigation is as shown in Table 3. From the result, serum high density lipoprotein cholesterol (HDL-c) concentration in diabetic control rats which decreased non-significantly when compared with the non-diabetic control (NC) was increased in all diabetic treatment groups including the insulin group (INS). These increases were however non-significant (P>0.05). Serum concentrations of total cholesterol (TC) (155.47±2.76), triacylglycerol (TG) (137.88±5.91), very low density lipoprotein cholesterol (VLDL-c) (27.54±1.15) were significantly increased (P<0.05) in DC group when compared with the NC. Treatments with extracts of SM showed a significant decrease (P<0.05) in TG (58.00±2.00), TC (35.00±1.00), VLDL-c (16.90±0.30), low density lipoprotein cholesterol (LDL-c) (14.70±0.53) when compared with DC group.

DISCUSSION

Changes in serum lipids concentration in mg/dl following treatment, in this investigation is as shown in Table 3. From the result, serum high density lipoprotein cholesterol (HDL-c) concentration in diabetic control rats which decreased non-significantly as compared to NC was increased in all diabetic treatment groups including INS. These increase were however non-significant (P>0.05). Serum concentrations of TC (155.47±2.76), TG (137.88±5.91), and VLDL-c (27.54±1.15) were significantly increased (P<0.05) in DC when compared with the non-diabetic normal control group (NC). Treatments with extracts of SM showed a significant decrease (P<0.05) in TG (58.00±2.00), TC (35.00±1.00), VLDL-c (16.90± 0.30), LDL-c (14.70±0.53) when compared with DC group.

Type 2 diabetes is commonly associated with dyslipidaemia which is a risk factor for the development of CVD (Koffi et al., 2009). This is supported by our present study. From this study, there was a marked increase in the lipid content of serum in streptozotocin induced diabetic rat. This is due to the increased mobilization of free fatty acid (FFA) from peripheral depot (Krishnaveni et al., 2010). Interestingly, most of the studies with different plant extracts in diabetic rats were in agreement with our result (Ladan et al., 2007). The rise in serum TG, TC, VLDL, LDL and low HDL in this study indicate derangement of lipid metabolism and amplified incidence of cardiac dysfunction in diabetic rats. Insulin deficiency or resistance may be responsible for dyslipidaemia, because insulin has an inhibitory action on HMG-COA reductase, a key enzyme which is responsible for the metabolism of LDL particles rich in cholesterol. Administration of extracts of *S. monostachyus* showed significant decrease (p<0.05) in TC, LDL, VLDL, and TG. This alleviation denotes the anti-hyperlipidaemic potential of *S. monostachyus*.

S. monostachyus in reversing the elevation of LDL-c, TC, VLDL-c, TG and decreased HDL-c in hyperlipidaemic rats agrees with the findings of Adaramoye et al. (2005) for diabetic and hyperlipidaemic rats. The crucial risk factor for CVD includes a low level of HDL-c. The association between a low level of HDL-c and an increased risk of CVD has been well established through epidemiological and clinical studies. LDL-c is a primary target of CVD risk reduction therapy. LDL-c transports cholesterol mainly to the arterial wall. This results in the build-up of insoluble lipid on the wall of the arteries thereby reducing blood flow and increasing the pressure on the arterial wall as well as the heart. The deposition of the cholesterol on the arterial wall results in a condition known as arteriosclerotic plaque which is the major cause of CVD. CVD are the leading cause of death in developing countries (Latunde Dada, 1990). Hypercholesterolaemia has been identified as a primary risk factor in the development of CVD. This implies that, preventing or reducing the serum levels is associated with reducing risk of CVD. In contrast, HDL plays a direct role in the atherogenic process. Therapeutic intervention by raising HDL-c is widely encouraged. In this study, SM led to a significant increase of HDL-c, indicating its promising

protective role against CVD. The protective role has been suggested to occur in various ways, HDL-c exert part of its anti-atherogenic effect by counteracting LDL oxidation. Recent studies also show that HDL promotes the reverse cholesterol transport pathway by inducing an efflux of excess accumulated cellular cholesterol and prevent the generation of an oxidatively modified LDL (Yokozawa et al., 2006). Furthermore, HDL inhibits the oxidation of LDL by transition metal ions, but also prevents 12-lipooxygenase-mediated formation of lipid hydro-peroxides. On the basis of the results of this study, SM may probably play an anti-atherogenic role through the inhibition of lipid oxidation as well as the elevation of HDL-c. People with higher levels of HDL-c seem to have fewer problems with CVD, while those with low HDL-c have increased rate of CVD. Some reports have shown that flavonoids, tannins and coumarin may play some role in antioxidant and hypolipidaemic effect (Ezekwe and Obidoa, 2001). The action of the plant extract in reducing plasma cholesterol concentration could be due to the ability of one or more of the phytochemicals in the plant to activate the functioning enzymes of the rat responsible for cholesterol absorption.

Conclusion

The results of this study show that the extract of SM can bring down the glucose levels in diabetic rats. Glucose levels of SM treated group decreased significantly when compared with the diabetic control group. The serum lipid profile level also decreased significantly when compared with the diabetic control group. Hence, we have come to the conclusion that this plant will be useful in the fight against diabetes.

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Conflict of interest

Authors have not declared any conflict of interest.

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