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Received 27 Jan, 2014: Accepted 30 May, 2014

Hybanthus enneaspermus is used by traditional birth attendants (TBAs) in southwest Nigeria in antenatal care. We examined the effects of oral administration of aqueous leaf extract of H. enneaspermus (HEaq) on liver weight and assay, maternal-thyroid hormones, foetal growth and litters birth weights in Sprague-Dawley (SD) rats. Twelve pregnant SD rats were used for this study. Control group received distilled water while the test group received 2 g/kg body weight of HEaq orally throughout the period of pregnancy. On day 19 of pregnancy, animals were sacrificed by CO₂ asphyxiation and cervical dislocation. Blood samples were withdrawn into sterile tubes and centrifuged to obtain serum that was used for assay. Liver homogenate was used for liver function test. The following were assayed; alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT), thyroid hormone, thyrotrophin and TSH in both control and treated rats. Some rats were allowed to carry their pregnancy to term. All rules guiding animal care and humane handling methods of laboratory animals in animal experimentation were followed. The results of the study showed a significant increase in serum and liver homogenate level of biomarkers of liver injury (ALP, ALT and AST). The serum T3 and TSH were significantly lowered in all extract treated rats compared to control, while T4 and thyrotrophin were not significantly altered. A significant reduction in placenta and foetal birth weights were recorded in the extract treated group. Oral administration of HEaq during pregnancy adversely affected maternal thyroid hormones, placental and foetal development. HEaq administration to pregnant rats also precipitated increased biomarkers of liver damage.

Key words: Hybanthus enneaspermus, pregnancy outcome, thyroid hormone, litters birth weight, liver function test.

INTRODUCTION

The activities of the traditional birth attendants (TBAs) are still common in Nigeria society where they attend to > 50% of deliveries (Oruamabo, 2007). One of the tools used by the TBAs is medicinal plant. Hybanthus enneaspermus is one of the most important medicinal plants used in southwest Nigeria by the TBAs (Awobajo et al., 2013). The TBAs claimed that administration of the concoction of the leaf extract of the H. enneaspermus
(HEaq) plant invigorate the pregnant woman, boost the haematological parameters and make delivery easy and safe (Awobajo et al., 2013). However, these claims are not scientifically verified. Several other medicinal plants have been shown to possess some advantages while many others have deleterious effects on body system (Dahanukar et al., 2000).

_H. enneaspermus_ (Linn) F. Mull commonly called “Abiwere” in Yoruba land, south west Nigeria is a shrub that belongs to the Violaceae family. We have earlier reported on the identification, extraction, phytochemistry and lethal dose (LD50) determination of the leaf extract of the plant (Awobajo et al., 2009a). Analysis of the chemical constituent has been reported (Anand and Gokulakrishnan, 2012). Other reported activities includes antimicrobial activities against some urinary tract pathogens (Sahoo et al., 2006; Awobajo et al., 2009), anticonvulsant and free radical scavenging activity (Hemalatha et al., 2003), antiplasmodial activity (Weniger et al., 2004), antidiabetic and antioxidant potentials (Patel et al., 2011). The oral infusion of the aqueous leaf extract of HEaq to pregnant rats precipitated poor pregnancy outcome (Awobajo et al., 2013). The aim of this present study was to further investigate the effects of HEaq on thyroid hormone and liver toxicity when administered orally to pregnant rats.

**MATERIALS AND METHODS**

**Animal grouping and treatment**

Two groups (control and HEaq) of mature female Sprague-Dawley rats weighing 170 to 180 g (12 rats per group) and four adult male rats were used for this study. They were housed in standard cages (4 rats/cage) at room temperature and provided clean water and rat chow _ad libitum_ throughout the experiment. Pregnancy was established by the presence of sperm cells in the vagina smear of normal cycling rats after 24 h cohabitation with adult male rat. The day sperm cells were discovered in the smear taken as day one of pregnancy. HEaq treated group were force-fed at a dose of 2 g/kg body weight (Awobajo et al., 2013) throughout the gestation duration, while the control rats received equal volume of distilled water; the vehicle. We have reported the method of identification, extraction and LD50 (LD50: 8.14 ± 0.30 or 8.24 ± 0.35 g/kg body weight) determination of the HEaq in previous publication (Awobajo et al., 2009a). Weekly weights of all animals were monitored.

**Blood collection and assay procedures**

On pregnancy day 19, half the numbers of rats in each group were sacrificed by cervical dislocation after CO2 asphyxiation. Blood samples were collected via cardiac puncture and centrifuged at 1500 g for 15 min to obtain the serum used for various assays. The following assays were carried out; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and thyroid hormones assay. AST, ALT, ALP was measured using commercially available kits ELISA kit and a UV-spectrophotometer (Hitachi 736-60, Japan). Hormonal assay was carried out with Randox assay kit, (UK) according to manufacturer specification. The liver was dissected out over ice pack, homogenized in phosphate buffer after which it was centrifuged at 1500 g for 15 min. The supernatant from liver homogenate and serum from blood samples were stored frozen at -8°C until used for the assays.

**Pregnancy outcome**

The remaining rats from each group were allowed to carry the pregnancy to term. Weights of litter were taken few minutes after delivery. Foetal and placenta recovered from rats sacrificed on 19th day of pregnancy were also recorded after laparotomy.

**Statistical analysis**

Results were analysed using analysis of variance (ANOVA) and presented in Tables as Mean ± standard error of mean (SEM). Level of significance was placed at p ≤ 0.05. All experimental protocols adopted in this study including animals handling were approved by the University and are in compliance with International guidelines on care and use of laboratory animals in biomedical research.

**RESULTS**

The results of the weight gain during pregnancy showed no significant (at p ≤ 0.05) difference in maternal weight gain over the duration of the pregnancy between control and treated rats, neither was there any difference in the pregnancy duration (Table 1). Foetal weight, placenta weight and litter birth weights in HEaq treated group of rats were all significantly reduced (p ≤ 0.05) compared with control group as recorded on day 19 of pregnancy (Table 2). Maternal thyroid hormone level on day 19th of pregnancy showed a significant reduction in T3 and TSH. The T4 level also was decreased but the decrease recorded was not significant. The T4/T3 ratio was significantly increased in the extract treated group compared to control (Table 4). There were no significant change to the weight gained and duration of pregnancy in HEaq group and control rats (weight gained control; 40.00 ± 5.77, HEaq; 40.00 ± 5.52; gestational duration control and HEaq; 21.00 ± 0.32).

The results of the liver function test showed a significant increase and a significant decrease (p ≤ 0.05) in serum and liver homogenate level of AST, respectively in HEaq rats compared to control. ALT was also significantly increased.
Decreased in the serum while it was increased in the liver homogenate in pregnant rats exposed to HEaq. ALP was only significantly increased (p ≤ 0.05) in the serum of HEaq rats while its level in the liver homogenate was not different from the results recorded for the control group (Table 3).

### DISCUSSION

Several environmental contaminants and phytoestrogens are known to affect the normal growth and development of the foetus despite the fact that the genome regulates the various stages of the development. Medicinal plants contain several phytochemicals, some of which are known to mimic some hormones in the body (Hughes, 1998; Dweck, 2006). Through the interactions of these phytochemicals with various receptors found in the body, they act as agonist or antagonist in the regulation of various cellular activities. There are reports of potential abortifacient activities of some of these medicinal plants (Nath et al., 1992), while others have been reported to produce birth defects (Costa et al., 2012).

The present study on toxic effects of HEaq in pregnant rats revealed a significant increase in serum level of ALP and AST, and a significant increase in liver tissue level of ALT on pregnancy day 19 compared to control. Serum or liver homogenate level of ALT (gold standard biomarker), ALP and AST are established biomarkers of liver injury, routinely used in assessing liver functions (Schomaker et al., 2013). This result showed that although HEaq may have wide oral toxic safety margin (Awobajo et al., 2009a), its administration to pregnant rats precipitated toxic effects. This will be detrimental to the pregnancy which has already placed high demand on the maternal liver function. The relative liver weight was also increased significantly in the extract treated group, a sign of hypertrophy possibly as a result of increase detoxification activities.

Although, there were no significant effect on maternal weight gained during pregnancy; foetal weight and placenta weight at day 19 of pregnancy were significantly reduced in HEaq treated rats. This also translated into a significant reduction in the weights of the litter delivered at term by all HEaq treated rats. Foetal nutrition has a lot to do with functional placenta which serve not only as the channels through which nutrient and gases are exchanged between the maternal and foetal blood but also secretes some hormones for sustenance of pregnancy (Sanin et al., 2001). Other authors have also linked the incident of intrauterine growth restriction to reduced placenta size, volume and weight (Metzenbauer et al., 2002). While increase in placenta weight during pregnancy has been well established to initiate corresponding foetal weight gain (Sanin et al., 2001), increased maternal thyroxine level has also been shown to stimulate placenta growth (Spencer et al., 1993). Therefore, the decrease in placenta weight recorded in HEaq group might have been precipitated by the decrease in T3 level. Thus, the decreased placenta growth may have resulted in reduced foetal growth and the significant reduction in birth weights of litter born to all HEaq treated rats.

Going by the importance of thyroid hormone to normal growth and development of the foetus and the reported 8.8% hypothyroidism prevalence in Saharan Africa (Sidibe, 2007), one may conclude that the prevalence may be worsened by the use of such medicinal plants with antithyroid potentials during pregnancy. However,
Table 3. Effects of oral administration of aqueous leaf extract of *Hybanthus enneaspermus* to pregnant rats on maternal Thyroid hormones, TRH and Thyrotropin on day 19 of pregnancy.

<table>
<thead>
<tr>
<th>Group</th>
<th>T3 (nmol/l)</th>
<th>T4 (nmol/l)</th>
<th>T4/T3 ratio</th>
<th>TSH (µIU/ml)</th>
<th>TRH (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.76 ± 0.08</td>
<td>74.00 ± 1.17</td>
<td>26.85 ± 0.57</td>
<td>3.35 ± 0.64</td>
<td>26.55 ± 3.61</td>
</tr>
<tr>
<td>HEaq</td>
<td>1.77 ± 0.09*</td>
<td>70.14 ± 0.43</td>
<td>40.14 ± 2.54†</td>
<td>1.12 ± 0.26*</td>
<td>26.70 ± 7.35</td>
</tr>
</tbody>
</table>

*Significantly lowered compared to the control (p ≤ 0.05). †Significantly increased compared to control (p ≤ 0.05).

Table 4. Maternal serum and liver homogenate level of AST, ALT and ALP on day 19 of pregnancy in rats treated with aqueous leaf extract of HEaq compared with control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum</th>
<th>Liver homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>HEaq</td>
</tr>
<tr>
<td>Aspartate aminotransferase ‘AST’ (U/L)</td>
<td>177.50 ± 7.76</td>
<td>210.21 ± 48.99†</td>
</tr>
<tr>
<td>Alanine aminotransferase ‘ALT’ (U/L)</td>
<td>222.95 ± 37.21</td>
<td>36.30 ± 2.04*</td>
</tr>
<tr>
<td>Alkaline phosphatase ‘ALP’ (U/L)</td>
<td>80.43 ± 21.28</td>
<td>137.96 ± 1.71†</td>
</tr>
</tbody>
</table>

*Significantly lowered compared to the control (p ≤ 0.05). †Significantly increased compared to control (p ≤ 0.05).

Further studies will be required to establish the mechanism via which HEaq produced alteration in placenta development and a possible link to the reduced foetal growth.

**Conclusion**

Oral administration of aqueous leaf extract of *H. enneaspermus* at a dose of 2 g/kg body weight to pregnant Sprague-Dawley rats increased risk of liver damage, precipitated reduction in placental growth and a reduction in maternal T3 and TSH with resultant reduction in litter birth weights. There is need to properly evaluate the merits and demerits of the various medicinal plants used by TBAs in care of pregnant women to ascertain their safety.

**Conflict of Interests**

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

**REFERENCES**


African medicinal plant derived products as therapeutic arsenals against multidrug resistant microorganisms

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Received 16 November, 2013; Accepted 5 May, 2014

Infectious diseases due to resistant pathogenic strains are rampant and the burden is worsened by the emergence and spread of microorganisms resistant to cheap and effective first-choice drugs. Medicinal plants could be an alternative solution to this and the aim of the present review is to summarize available evidence and knowledge concerning African medicinal plants used to treat multidrug resistant (MDR) bacteria, fungi and protozoa infectious agents. A literature search using the keywords: Africa, medicinal plants and multi-resistant microorganisms on google scholar, African Index Medicus, PubMed, Medline and EMBASE was conducted. We also scanned reference lists for important citations. Key pharmaceutical journals, workshop and conference proceedings were reviewed. Common medicinal plants found are Brueca javanica, Prunus Africana, Mangifera indica, Picralima nitida, Aloe arborescence, Aloe striata, Vernonia adoensis, Markhamia tomentosa, Garcinia lucida, Garcinia kola, Phyllanthus muellerianus, Gladiolus gregasius, Sida alba, Trichila heudelotti, Piptadeniastrum africana and Dorstenia picta. Most researches on the use of medicinal plants to treat multidrug resistant agents were conducted in South Africa, Nigeria, Cameroon, Congo, Kenya, Zimbabwe, Burkina Faso and Uganda. African medicinal plants possess important therapeutic agents that can be used as new phyto-medicines against MDR microorganisms.

Key words: Africa, medicinal plants, therapeutic products and multidrug resistant microorganisms.

INTRODUCTION

The spread of microorganisms which are resistant to cheap and effective first-choice drugs, although a natural phenomenon, is becoming a public health concern. Favorable factors accounting for this include auto-medication, treatment outside of recognized treatment centers and consumption of drugs without medical supervision and during insufficient length of time. Other confounding factors include frequent movements of population, overcrowding which provides opportunities for the rapid spread of microorganisms including multidrug...
resistant strains (Njunda et al., 2012). Multidrug resistance to as many as seven antibiotics has been observed in many epidemiological studies in Africa (Iruka and Sosa, 2008; Njunda et al., 2012). Antibiotics that usually face resistance are ampicillin, amoxicillin, cotrimoxazole, gentamicin, ceftriaxone and augmentin (amoxicillin + clavulanic acid) because of their wide indiscriminate use. Microorganisms that usually cause antibiotic failure include those that affect the urogenital tract such as beta-lactamase producers Neisseria gonorrhoeae, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, methicillin resistant Staphylococcus aureus (MRSA) and mycoplasmas (Njunda et al., 2011). Those responsible for pulmonary infections include Mycobacterium tuberculosis, Klebsiella pneumonia and Streptococcus pneumonia. Agents responsible for gastrointestinal infections include Escherichia coli, Entamoeba histolica, Salmonella typhi and Shigella dysenteriae (Njunda et al., 2011).

Various mechanisms to acquire or develop intrinsic resistance to antibiotics which are developed by pathogens include active efflux of drugs, alteration of target sites, enzymatic degradations and propagation of resistance genes (Sibanda and Okoh, 2007). Bacterial resistance to penicillin is expanding to cephalosporins leading to the development of plasmid-mediated extended spectrum β-lactamases (ESBLs) strains. As new antimicrobial compounds are discovered, there is a need to assess their potentials in combination therapies with old antibiotics that have been rendered ineffective by the development of resistant strains (Sibanda and Okoh, 2007).

Management of infectious diseases caused by beta-lactam-resistant bacteria strains in developing countries where efficient antibiotics are not affordable for the majority of the population is becoming urgent and alternative agents can be obtained from medicinal plants. In Africa, medicinal plants are used in the fight against many infectious ailments; they still play a great role as therapeutic agents in many African countries (Kuete et al., 2011). They may form a good source of antimicrobial medications or resistance modifying agents to be discovered. Treatment of endemic infections using available natural resources would provide more efficient drugs to patients. Many medicinal plants and their derived products now form part of the therapeutic arsenals of Africa, faced with ever increasing infectious diseases, emerging and re-emerging infectious diseases due to multidrug resistant microorganisms and immune deficient diseases such as HIV/AIDS. It is estimated that more than 5,000 active principles have been identified in fruits, vegetables and grains, but a large percentage still remain unknown and need to be studied to increase our understanding of their health benefits (Kuete and Effert, 2010).

Studies on the antimicrobial properties of medicinal extracts on resistant strains of microorganisms are scanty and only few antimicrobial agents as isolated compounds have been proven to possess inhibitory properties on multidrug resistant microorganisms. However, phytomedicines as antimicrobial agents have been evaluated scientifically in various countries in Africa. They present a low risk of resistance development to their action, because they are complex mixtures, making microbial adaptability very difficult (Daferera et al., 2003). This review summarizes the currently available knowledge on medicinal plants used to treat multidrug resistant infections and the efficacy of plant-derived extracts and compounds across Africa.

METHODOLOGY

A literature search using the keywords: Africa, medicinal plants and multi-resistant microorganisms on Google scholar, African Index Medicus, PubMed, Medline and EMBASE was conducted. We also scanned reference lists for important citations. Key pharmaceutical journals, workshop and conference proceedings were reviewed. African researchers of medicinal plants were contacted.

RESULTS

Plant compounds with activity against multi-resistant bacteria pathogens in Africa

Some isolated pure compounds of plant origin have been reported to have resistance modifying activities in vitro. This has prompted the search for such compounds from a variety of medicinal plants. Some of the compounds which have been observed to have direct antimicrobial activity were also able to potentiate the activity of antibiotics when used at low minimum inhibitory concentration (MIC) levels. For instance the antimicrobial property of tea (Camellia sinensis) is due to polyphenols (Kim et al., 2000). Bioassay directed fractionation of its extracts revealed its content in bioactive components such as epicatechin gallate (ECG), epigallocatechin gallate (EGCG), epicatechin (EC) and caffeine (CN). Used in combination in vitro, ECG and CG reduced MIC values for oxacillin from 256 and 512 to 1 and 4 mg/L against methicillin resistant Staphylococcus aureus (MRSA) (Kim et al., 2000). Ethyl gallate is a congener of alkyl gallates, purified from a dried pod of tara (Caesalpinia spinosa) native to South America, intensified beta-lactam susceptibility in MRSA and MSSA strains (Kim et al., 2000).

Twenty six species of medicinal plants belonging to 19 families were described in previous studies were shown to possess antimicrobial properties on multidrug resistant bacteria species in six different countries across Africa (Table 1). These plants are used in traditional folk medicine against all types of infectious ailments including gastrointestinal, pulmonary, genito-urinary tracts and skin and skin infections. Seeds, stem barks, leaves, bulbs, twigs and roots are the plants’ parts extracted using mostly methanol, water and acetone as major solvents. Although these studies indicated inhibitory activities on...
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<tr>
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<th>Country</th>
<th>Traditional treatment</th>
<th>Plant parts used</th>
<th>Solvent used/screened activity</th>
<th>Bioactive compounds</th>
<th>Microbial strains</th>
<th>Antibiogram resistance profile</th>
<th>References</th>
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<tr>
<td>Anacardiaceae</td>
<td>Mangifera indica</td>
<td>Zimbabwe</td>
<td>cough and diarrhea</td>
<td>twigs and leaves</td>
<td>Ethanolic extract, efflux inhibitory activity of extract comparable to reserpine</td>
<td>tannins, phenols, alkaloids, glycosides</td>
<td>K. pneumonia; S. aureus; P. aeruginosa; Bacillus cereus</td>
<td>-</td>
<td>Chitemerere and Mukanganyama 2011</td>
</tr>
<tr>
<td>Apocynaceae</td>
<td>Picralima nitida (Stepf.) T. &amp; H.Durand</td>
<td>Cameroon</td>
<td>Hypertension, fever, malaria, anti-inflammatory, antimicrobial</td>
<td>Leaves, seeds</td>
<td>Methanolic: 1.25 &lt; MIC ≤ 10 mg/ml</td>
<td>-</td>
<td>E. coli 25922, E. aerogenes</td>
<td>-</td>
<td>Gangoué-Piéboji et al., 2009</td>
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<tr>
<td>Ashodelaceae</td>
<td>Aloe arborescens</td>
<td>South Africa</td>
<td>Treatment of diarrhoea and stomach ailments</td>
<td>Leaves</td>
<td>Acetone. C = 0.078 mg/ml</td>
<td>TLC fingerprint revealed the presence of flavonoids and triterpenoids</td>
<td>S. typhimurium</td>
<td>ESBL positive Amx, Amp, aztreonam, Pip/tazobactam, COT Oxazole and Tet with reduced susceptibility to Cl</td>
<td>Bisi-Johnson et al., 2012</td>
</tr>
<tr>
<td></td>
<td>Aloe striata;</td>
<td>South Africa</td>
<td>Treatment of diarrhoea and stomach ailments</td>
<td>Leaves</td>
<td>Acetone. C = 0.078 mg/ml</td>
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<td>Asteraceae</td>
<td>V. adoensis</td>
<td>Zimbabwe</td>
<td>boiled decoction active against TB</td>
<td>Leaves</td>
<td>Ethanolic extract, Efflux inhibitory activity of extract with reserpine as reference</td>
<td>-</td>
<td>K. pneumonia, S. aureus, P. aeruginosa, B. cereus</td>
<td>-</td>
<td>Chitemerere and Mukanganyama 2011</td>
</tr>
<tr>
<td>Bignoniaceae</td>
<td>Markhamia tomentosa (Benth) K. Schum</td>
<td>Nigeria</td>
<td>Anti snake venom/bite, sore eyes, heart pain, scrotal elephantias</td>
<td>Leaves</td>
<td>methanolic extract</td>
<td>-</td>
<td>S. aureus; NCTC6571 B. subtilis ; NCIB 3610 P. aeruginosa. ATCC 10145</td>
<td>PV CE EM ; Aug Tet PV CE EM ; NaI PV CE EM</td>
<td>Aladesanmi et al., 2007</td>
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<td>Clusiaseae</td>
<td>Garcinia lucida</td>
<td>Cameroon</td>
<td>Gastric ulcer, fermentation of palm wine, gynaecological infections, anti-poison, gastrointestinal infections, snake bites</td>
<td>Seeds, stem bark</td>
<td>Methanolic: 1.25 &lt; MIC ≤ 10 mg/ml</td>
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<td>Uses</td>
<td>Part Used</td>
<td>Extraction Method</td>
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<td>E. coli, K. pneumonia, A. baumannii</td>
<td>Gangoué-Piéboji et al., 2009</td>
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<td>Phyllanthus muellerianus</td>
<td>Cameroon</td>
<td>Use for infectious ailments</td>
<td>Stem barks</td>
<td>Methanolic</td>
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<td>All resistant against Amp Augmentin</td>
<td>Assob et al., 2011</td>
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<td>Bridelia micantha (Hochst.) Baill.</td>
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<td>Cough, antimicrobial, diarrhea, gastric ulcer, intestinal worms, eye diseases</td>
<td>Stem barks</td>
<td>Methanolic</td>
<td>1.25 &lt; MIC ≥ 10 mg/ml</td>
<td>-</td>
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</tr>
<tr>
<td>Dorstenia picta</td>
<td>Cameroon</td>
<td>Diarrhoea, infected wounds, anti-inflammatory, antimicrobial, eye diseases, snake bites</td>
<td>Leaves</td>
<td>Methanolic</td>
<td>1.25 &lt; MIC ≥ 10 mg/ml</td>
<td>-</td>
<td>E. coli, K. pneumonia, A. baumannii</td>
<td>Gangoué-Piéboji et al., 2009</td>
<td></td>
</tr>
<tr>
<td>Moraceae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorstenia Bateri</td>
<td>Cameroon</td>
<td>Twigs</td>
<td></td>
<td>CH2Cl2/MeOH (1:1) or in MeOH, 5 &lt; MIC &gt; 36 µg/ml</td>
<td></td>
<td>Flavoids: isobachalcone (IBC), kanzanol C (KAN), 4-hydroxylonchocarpin (4-LCP), stipulin (SPL), amentoflavone (AMF)</td>
<td>K. pneumonia, S. aureus, P. aeruginosa</td>
<td>Nuonrrohoea NGCSS (_L+) Kuete et al., 2010</td>
<td></td>
</tr>
<tr>
<td>Psidium guajava</td>
<td>South Africa</td>
<td>Use in the treatment of diarrhoea and stomach ailments</td>
<td>Leaves</td>
<td>Acetone: C=0.312-0.625 mg/ml</td>
<td>TLC fingerprint revealed the presence of flavonoids and terpenoids.</td>
<td>S. Typhimurium ESBL positive Amx, Amp, aztreonam, Pip/tazobactam, COT Oxazol and Tet with reduced susceptibility to CI</td>
<td>Bisi-Johnson et al., 2012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calistermon citrinus Skeels</td>
<td>Zimbabwe</td>
<td>Antibacterial, hemorrhoid treatment</td>
<td>Leaves</td>
<td>Ethanol extract, efflux inhibitory activity of extract comparable to reserpine</td>
<td>-</td>
<td>K. pneumonia, S. aureus, P. aeruginosa Bacillus cereus</td>
<td>-</td>
<td>Chitemerere and Mukananganya ma 2011</td>
<td></td>
</tr>
</tbody>
</table>
### Table 1. Contd.

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>Country</th>
<th>Use for infectious ailments</th>
<th>Plant Part</th>
<th>Plant Part</th>
<th>Isolated Compounds</th>
<th>In Vitro Sensitivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iridaceae</td>
<td>Gladiolus</td>
<td>gregasius Baker</td>
<td>Cameroon</td>
<td>Use for infectious ailments</td>
<td>Bulbs</td>
<td>Methanolic</td>
<td>Sap Tan Gly CG RS</td>
<td>E. coli, K. pneumonia, P. aeruginosa, P. mirabilis, S. flexneri, S. typhi</td>
<td>Assob et al., 2011</td>
</tr>
<tr>
<td>Malvaceae</td>
<td>Sida</td>
<td>alba L.</td>
<td>Burkina Faso</td>
<td>Use in treating infectious diseases in children, malaria, fever, pain, variolae, antibacterial, anti-inflammatory, analgesic activities and hepatoprotective</td>
<td>Leaf stems</td>
<td>Aqueous/acetone (80%, v/v), Synergistic effect when polyphenol rich fractions are combined to COT</td>
<td>Polyphenols</td>
<td>Shigella dysenteriae, Shigella boydii, Enterococcus faecalis, Proteus mirabilis</td>
<td>All resistant to COT</td>
</tr>
<tr>
<td>Meliaceae</td>
<td>Trichila</td>
<td>heudelotti</td>
<td>Nigeria</td>
<td>Sores, heart troubles, pile</td>
<td>Leaves</td>
<td>Methanolic extract ID obtained at 250 mg/ml</td>
<td>-</td>
<td>E. coli NCTC 10418, S. aureus, NCTC6571, B. subtilis, NCIB 3610, P. aeruginosA. ATCC 10145</td>
<td>Nal Aug Tel Amx CL, PV CE EM, Amp PV CE EM, Aug Tel PV CE EM, Nal PV CE EM</td>
</tr>
<tr>
<td>Mimosaceae</td>
<td>Piptadeniastrum</td>
<td>africana</td>
<td>Cameroon</td>
<td>Use for infectious ailments</td>
<td>Leaves</td>
<td>Methanolic</td>
<td>Fla Pol Cou Gly CG RS</td>
<td>E. coli, K. pneumonia, P. aeruginosA, P. mirabilis, S. flexneri, S. typhi</td>
<td>All resistant against Amp Augmentin</td>
</tr>
<tr>
<td>Nyctaginaceae</td>
<td>Boerhavia</td>
<td>diffusa</td>
<td>Nigeria</td>
<td>Diabetes, anti inflammatory, Abscess, boils</td>
<td>Leaves</td>
<td>methanolic</td>
<td>-</td>
<td>B. subtilis NCIB 3610</td>
<td>Aug Tet PV CE EM</td>
</tr>
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</table>
activities on many gram+ and gram- multidrug resistant bacteria strains, only few presented the phytochemical composition of these plants. Alkaloids, flavonoids, phenols and triterpenes appear to be the main compounds identified. Microbial strains that dominated in the studies were Escherichia coli, K. pneumonia, S. dysenteriae, E. faecalis, Salmonella typhi, P. aeruginosa, S. aureus methicillin resistant and Bacillus subtilus, Neisseria gonorrhea beta lactamase+. They were found to be resistant to a wide range of antibiotics including amoxicillin, ampicillin, augmentin, cephaplatin, ciprofloxacin, ofloxacin, cotrimoxazol, methicillin. Particularly of interest, the study done in Burkina Faso on the aqueous acetone (80%) of Sida alba L., a synergistic effect was observed when polyphenol rich fractions of this plant were combined to cotrimoxazole against Shigella dysenteriae, Enterococcus faecalis and Proteus mirabilis (Konaté et al., 2012). This is an indication that polyphenols could act in combination to cotrimoxazole for potentialization in order to increase its efficacy.

In Cameroon, the studies focused on beta-lactam-resistant bacteria (K. pneumoniae, K. oxytoca, Enterobacter cloacae, Serratia marcescens, Acinetobacter baumannii, S. aureus and Enterococcus sp.) and reference strains of bacteria (E. coli ATCC 35218, Enterobacter aerogenes ATCC 29751, E. aerogenes ATCC 13048, P. aeruginosa ATCC 27853 and Enterococcus hirae ATCC 9790) by using disc-diffusion and agar-dilution assays (Gangoué-Pieboji et al., 2009). The minimal inhibitory concentration (MIC) values of different plant extracts against the tested bacteria were found to range from ≤ 0.3 to ≥ 10 mg/ml. This study revealed that the most active plant extracts were Dorstenia picta and Bridelia micrantha on beta-lactam-resistant gram-negative bacilli and the extracts from Bridelia micrantha, Mallotus oppositifolius, Garcinia lucida, Garcinia kola, Campylospermum densiflorum (leaves) and Campylospermum zenkeri (root) on beta-lactam-resistant gram-positive cocci (Gangoué-Pieboji et al., 2009). Another innovative approach in searching for new arsenals against multi-resistant bacteria was seen in the study carried out in Zimbabwe by Chitemerere and Mukanganyama (2011), who found that the ethanolic leaves extract of Callisteron citrinus Skeels rich in tannins, phenols, alkaloids and glycosides showed an efflux inhibitory activity against S. aureus, K. pneumonia, P. aeruginosa and B. subtilis comparable to that of reserpine. Reserpine is an alkaloid and is a reference efflux inhibitor with a useful counteracting mechanism against microorganisms’ resistance. Antimicrobial activity of Temnocalyx obovatus (Rubiacae) root extracts used in folk medicine in Zimbabwe deployed significant activity against bacterial (S. aureus, E. coli, Clostridium perfringens), and fungal (Aspergillus niger and Candida albicans) species with MIC values ranging from 10 to 60 μg/ml(Muna and Fauzia, 2012).

Other efflux inhibitory mechanisms were observed by Aiyegoro et al. (2009) who showed that the activity of presumed plant antimicrobials against gram+ and gram- organisms was significantly
enhanced by synthetic multidrug-resistance (MDR) inhibitors of MDR efflux proteins. Combinations of antibiotics are progressively being used in the treatment of drug resistant infections; this is advantageous because they deploy various mechanisms of action. The production of intrinsic antimicrobial compounds by plants can have a MDR inhibitory property that improves the inhibitory effect of antibiotics (Aiyegoro et al., 2009). The use of Catha edulis extracts for instance at sub-inhibitory levels was reported to reduce the MIC values of tetracycline and penicillin G against resistant oral pathogens, Streptococcus oralis, Streptococcus sanguis and Fusobacterium nucleate. Polyphenols (epicatechin gallate and catechin gallate) were also found to be able to reverse beta-lactam resis-tance in methicillin resistant S. aureus (MRSA). Whereas diterpenes, triterpenes, alkyl gallates, flavonoids and pyri-dines showed resistance modulating abilities on various antibiotics against resistant strains of S. aureus (Aiyegoro et al., 2009). The methanolic and ethylacetate extracts of Phyllanthus muellerianus and Piptadeniastum africanus were found to be highly active against gram+ and gram- infectious resistant microorganisms with MIC varying from 2.5 to 0.31 mg/L. The in vivo acute toxicity study carried out on the methanolic extracts of these two plants indicated that they were not toxic (Assob et al., 2011). This is important because selective inhibition is crucial to conclude on the efficacy of antimicrobial compounds.

Other studies from Cameroon on plant extracts showed interesting results due to their exceptional inhibitory power on both bacteria and fungi. Among these are Bersama engleri ana, Dorstenia angusticornis, Dorstenia barteri, Diospyros canaliculata, Diospyros crassiflora, Newbouldia laevis, and Ficus cordata (Kuete et al., 2010). Compounds like isobavachalcone, kanzanol C and 4-hydroxylorenchocarp isolated from Dorstenia spp., plumbagin, crassiflorone and diospyrone isolated from Diospyros spp., and also new boudiaquinone and lapachel isolated from Newbouldia laevis (16) displayed important inhibitory activity against resistant microorganisms. Thecacoris annobonae Pax & K. Hoffm (Euphorbiaceae) has a significant antimicrobial (MIC < 10 μg/ml) activity against Mycobacterium tuberculosis H37Rv, B. cereus and P. aeruginosa (Kuete et al., 2011). The effect of combinations of the methanolic extract of Helycrismus pendunculatum leaves and selected antibiotics evaluated using the time-kill assay method showed a synergy rate of 59.1% (Extract + Tetracycline; Extract + Amoxycillin), 54.6% (Extract + Penicillin G; Extract + Chloramphenicol), 63.6% (Extract + Ciprofloxacin; Extract + Oxytetracycline), 68.2% (Extract + Erythromycin) and 27.3% (Extract + Ampicillin) on all isolates at both ½ MIC and MIC values. Overall, synergistic response could attain 60% of all combinations of extract and antibiotics against all tested organisms (Aiyegoro et al., 2009).

In Libya, antituberculosis activity was obtained with dichloromethane extract of Tubagahia violacea Harv. against M. aurum; Marrubium vulgare L., Pistacia lentiscus, Quercus coccifera L, Thymus capitatus (L.) Hoffm. & Link are active against M. tuberculosis (Aiyegoro et al., 2009; Korir et al., 2012).

Plant compounds with efficacy against multi-resistant fungi pathogens in Africa

A good number of studies have been done on the efficacy of medicinal plants against fungi. In Nigeria, Spen oceutrum jollyanum Pierre (Menispermaceae) used in traditional medicine as chewing sticks and for stomach pains was shown to possess significant antifungal activity against Candida albicans, Candida pseudotropicalis and Trichophyton rubrum. In South Africa (Aladesanmi et al., 2007), extract of the rhizomes Gunnera perpensa was proven effective against Penicillium notatum, Aspergillus flavus and A. niger (LC50 values ranging from 0.07 to 3.81). This is important as these fungi have been implicated in cases of immune-compromised patients that frequently develop opportunistic and superficial mycosis (Nkomo and Kambizi, 2009).

An important antifungal activity was obtained with the acetone bark extract of Erythrina caffra Thunb. The bark of E. caffra is used in South Africa to treat sores, tuberculosis, respiratory infections, wounds, abscesses, arthritis and toothache. Dose dependent inhibitory activity was observed against a wide range of fungi strains (Candida krusei, C. albicans, Candida neoforms, Candida rugosa, P. notatum, A. niger, Aspergillus terreus, Aspergillus flavus, Absidia corymbifera, Candida glabrata, Trichophyton mucoides, Trichophyton tonsurans and Fusarium Sporotrichoïdes) (Nkomo and Kambizi, 2009) with MIC and minimum fungicidal concentrations (MFC) values ranging between 0.625 and 20 mg/ml and indicating fungicidal activity (Olajuyigbe and Afolayan, 2012). In Cameroon, another study presented the efficacy of Ficus polita (Moraceae) against C. albicans (Kuete et al., 2011). The bark and roots infusions are used in the treatment of infectious diseases, abdominal pains and diarrhea. Phytochemical analysis of the plant indicated the presence of compounds with enough evidence of their antimicrobial microbial activities such as lupeol, betulinic acid, ursolic acid, b-sitosterol, sitosterol-3-O-b-D-glucopyranoside (Kuete et al., 2011).

Cryptolepis sanguinolenta Lindl. Schitr. (Periploca ceae) is a shrub that grows in the rainforest and the deciduous belt forest, found in the west coast of Africa. In vitro study showed inhibitory activity against bacteria species (specifically, enteric pathogens, most notably E. coli and vibrio) as well as against Candida spp. (Iwu et al., 1999). Aframomum melegueta (Zingiberaceae) is a perennial herb used as an aphrodisiac and against measles and leprosy, taken for excessive lactation and post partum
hemorrhage, purgative, galactogogue and anthelmintic and hemostatic agent contains gingerol, shagaol, paradol in its essential oil; its antifungal antishistosomal activities have also been demonstrated (Iwu et al., 1999; Kuete et al., 2011). Xylopic acid, one of the constituents of *Xylopia aethiopica*, Ethiopian Pepper (Abbibaceae) is active against *C. albicans* (Kuete et al., 2011; Korir et al., 2012). Some extracts from Cameroonian medicinal plants including those from *Bersama engleriana*, *Dorstenia angusticornis*, *Dorstenia barteri*, *Diospyros canaliculata*, *Diospyros crassiflora*, *Newbouldia laevis* and *Ficus cordata* exhibited a wide range of inhibitory activity on both bacteria and fungi (Kuete et al., 2011). In Kenya, hexane extracts of *Senna didymobotrya* used in folk medicine showed notably inhibitory activities on *Microsporum gypseum*, *Trichophyton mentagrophyte* and *Microsporum gypseum* (Omoregie and Sisodia, 2012).

Plants with anti-malarial and other anti-protozoa multi-resistant efficacy

Malaria is a serious health concern in Sub-Saharan Africa where it kills a child below five years every thirty seconds and more than 90% of deaths occur due to this pathology (Nsagha et al., 2011). Anti-malarial multidrug resistance is a major public health problem in the world especially in Africa where the health systems are weak resulting in many prescriptions by unqualified health personnel (Nsagha et al., 2012) and home-based treatment (Htut, 2009) which affects its control. Over the years, anti-malaria drug resistance has become one of the most important problems impeding malaria control efforts (Sendagire et al., 2005). This led to the discovery of other antimalarial agents from medicinal plants such as quinine from cinchona bark and artemisinin from *Artemisia annua* (Saxena et al., 2003). One of such plants with anti-malarial and antiprotozoa potentials on the African continent is *Brueca sumatran a* Roxb., a shrub belonging to the family Simaroubaceae (Ehata et al., 2012). An important inhibitory activity was obtained in vitro with the seeds' crude extracts of *B. sumatran a* against *Trypanosoma cruzi*, *T. brucei brucei*, *Leishmania infantum* and chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum* in the Democratic Republic of Congo (Ehata et al., 2012).

Plants from Western Cameroon were screened in vitro for their antiplasmodial activity and cytotoxicity. *Dacryodes edulis* exhibited the highest antiplasmodial activity, followed by *Vernonia amygdalina*, *Coula edulis* and *Eucalyptus globulus*. *Dacryodes edulis* is a multi-purpose plant in African folk medicine, as its various parts are used as a remedy for parasitic skin diseases, jigger, mouthwash, tonsillitis, sickle cell and malaria (Zofou et al., 2011). Its phytochemistry revealed the presence of phenolic compounds which have previously been shown as having antiplasmodial activity (Zofou et al., 2011). An alkaloid, akuamine (Figure 1) from the seeds of *Picralima nitida* possesses activity against *Plasmodium* (Titanji et al., 2008). *V. amygdalina* and *Eucalyptus globulus* extracts exhibited high activity (*1 < IC_{50} < 10 \mu g/ml*) on both chloroquine sensitive and multidrug resistant strains of *P. falciparum* (*10 < IC_{50} < 25 \mu g/ml*) (Titanji et al., 2008).

Antiprotozoal activities of *Albizia zygia* (Fabaceae) stem bark and methanolic seeds' extract of *Harungana madagascarensis* was obtained against *P. falciparum* K1 chloroquine-resistant strain, *Leishmania donovani*, *Trypanosoma cruzi*, *Trypanosoma brucei rhodesiense*, protozoa responsible for malaria, visceral leishmaniasis, Chagas disease and African trypanosomiasis by Lenta et al. (2007). In Congo, it was found that the extracts from

![Figure 1. Akamine. Source: Titanji et al. (2008).](https://example.com/akamine.png)
Enanatia chlorantha stem bark, Napoleona vogelii stem bark and Quassia africana root bark are active with IC₅₀ values ranging between 1.87 and 5 µg/ml, against Trypanosoma cruzi, Leishmania infantum and P. falciparum K1 (Musum), 7α- obacunyl acetate and a cycloartane derivative which are isolated compounds from the dichloromethane - methanol (1:1) extract of the stem bark of Entandrophragma angolense (Meliaceae) with good activity, IC₅₀ of 2 and 5.4 µg/ml, respectively test against chloroquine resistant strain W2 of P. falciparum malaria parasite in Cameroon (Bickii et al., 2007; Sha’a et al., 2011). In Benin, two sesquiterpenic lactones isolated (1(15-acetoxy-8β)-(2-methylbutyryloxy)-14-oxo-4,5-cis-acanthospermolide) and 2 (9α-acetoxy-15-hydroxy-8β,(2-methylbutyryloxy)-14-oxo-4,5-trans-acanthospermolide) from the aerial parts of Acanthospermum hispidum D.C showed in vitro antiplasmodial activity against the chloroquine-sensitive strain (3D7) (IC₅₀ of 2.9 ± 0.5 and 2.23 ± 0.09 µM, respectively), Trypanosoma brucei brucei (IC₅₀ of 2.45 ± 0.49 and 6.36 ± 1.42 µM, respectively) and Leishmania mexicana mexicana (IC₅₀ of 0.94 ± 0.05 and 2.54 ± 0.19 µM, respectively) (Ganfon et al., 2012).

In Nigeria, the ethanolic extract of Jatropha tanjorensis leaves showed moderate sensitivity against P. falciparum. Alkaloids, saponins, anthraquinones, tannins and flavonoids are probably responsible for this activity (Ouattara et al., 2006; Omorogie and Sisodia, 2012). Ethanol extract of V. amygdalina induced an important inhibitory activity against P. falciparum with an IC₅₀ of 11.2 µg/ml (Ouattara et al., 2006; Sha’a et al., 2011). Whereas in Burkina Faso, methanolic extracts of Swartzia madagascariensis and Combretum glutinosum as well as alkaloidal extracts of Tinospora bakis were proven to be active against P. falciparum chloroquine-resistant strain W2 in vitro (5 µg/ml < IC₅₀ < 50 µg/ml) (Ouattara et al., 2006; Dzomba and Muchanyereyi, 2012).

**DISCUSSION**

In Africa, attitudes towards traditional, herbal medicines vary strongly because of the confusion between herbal medicine and witchcraft (Wright et al., 1988). The use of medicinal plants is often associated with superstition and therefore rejected by some people. However, there are many Africans who prefer traditional methods of treatment (Wright et al., 1993). The Chinese plant, Artemisia annua is cultivated in East African countries to supply pharmaceutical manufacturers in Europe (Wright et al., 1993). The bark of Prunus africana is used in making treatments for prostate cancer (Wright et al., 1993). Brueca sumatrana have been proven to possess antiprotozoal activity against Trypanosoma cruzi, T. brucei brucei, Leishmania infantum and chloroquine and pyrimethamine-resistant K1 strain of P. falciparum. These are in consonance with findings from the Asian species that were investigated for their various biological activities such as antimalarial, antiprotozoal against amoeba (Camacho et al., 2003; Sawangjaroen and Sawangjaroen, 2005), Toxoplasma gondii and Giardia intestinalis (Camacho et al., 2003), Trypanosoma brucei brucei and Leishmania donovani (Sawangjaroen and Sawangjaroen, 2005), Blastocystis hominis (Bawm et al., 2008), Trypanosoma evansi (Subeki et al., 2007) and Babesia gibsoni (Elkhateeb et al., 2008; Okokon and Nwafor, 2009). Other studies on medicinal plants have been reported from Cameroon (Lenta et al., 2007; Djeussi et al., 2013), Nigeria (Runyoro et al., 2006; Balagon et al., 2010), South Africa (Wright et al., 1993), Tanzania (Owuor and Kisangau, 2006), Uganda (Sendagire et al., 2005), Kenya (Mejhabeen et al., 2011) and Congo (Wright et al., 1988).

**CONCLUSION AND FUTURE PROSPECTS**

Several *in vitro* and *in vivo* antimicrobial activities have been carried out on traditional medicinal plants and found to be good sources of numerous therapeutic agents, however only few studies on the efficacy of medicinal plants and their derived compounds have been done so far on resistant strains of microorganisms. Many complementary and alternative medicines are being given more consideration because a large number of antimicrobial agents derived from traditional medicinal plants are available for treating various infectious diseases. In most of the studies, interesting antimicrobial properties of extracts against MDR strains were obtained, suggesting their potency, but only few studies have actually addressed the issue of selective toxicity.

Emphasis should be oriented towards the discovery of antibiotic resistance modifying compounds from plants sources which offer greater chances of reversing the resistance pattern of many antimicrobials which otherwise will be abandoned. Combined therapies of plants extracts and their derived products have to be assessed *in vivo* as well as to determine their clinical relevance. This will surely lead to the production of phytomedicines or medicinal plant-derived therapeutic agents. In order to attain this goal, research institutions in Africa should move further and put in place strong protocols geared towards the evaluation and comparison of the cost-effectiveness and safety-tolerance levels of the medicinal plants and their derived products against multidrug resistant microbial agents. African states should reinforce these efforts by putting in place a common policy that fosters the integration of traditional and modern medicines.

**Competing interests**

The authors declare that they have no competing interest
in this study.

ACKNOWLEDGEMENT

The authors acknowledge with thanks, the financial support from the Cameroonian Ministry of Higher Education through the Research Modernization scheme and the International Foundation for Science, for the research grant offered to the lead author.

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- Medical Practice and Reviews
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