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Antioxidant, DNA protective and antibacterial activities of *Terminalia bellerica* extracts

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***Terminalia bellerica* Roxb., commonly known as Beleric myrobalan, is a well-known large deciduous tree with various pharmaceutical properties. However, scientific information on *T. bellerica*, growing in India, as regards to its phytochemical constituents and pharmacological properties is very limited. With this in view, this study investigated the antioxidant, DNA protective and antibacterial activities of different parts (fruit pulp, seed and bark) of *T. bellerica*. Methanol (70%) and water (100% distilled water) were used for the extraction and analysis of total phenolic contents (TPC). Plant extracts were further screened for antibacterial activity against both gram (+) and gram (-) bacteria and minimum inhibitory concentration (MIC) values were calculated. Amongst the tested extracts, methanolic extract contained more TPC than aqueous extract. Methanolic fruit pulp (MEFP) showed lower IC_{50} (118.7 µg/ml) for free radical, (77.65 µg/ml) superoxide anion radical, (73.76 µg/ml) hydroxyl radical scavenging activity, (115.6 µg/ml) lipid peroxidation and (184.98 µg/ml) ferric thiocyanate assay. Methanolic extract also exhibited more potential DNA protective and antibacterial activity than aqueous extract. Furthermore, the correlation between TPC and antioxidant studies revealed that phenolics are mainly responsible for antioxidant, DNA protective and antibacterial activities of *T. bellerica*. This study suggests that the methanolic extract of *T. bellerica* could be a potential source of natural antioxidants.**

Key words: *Terminalia bellerica*, antioxidative, DNA protective, antibacterial.

INTRODUCTION

Reactive oxygen species (ROS) cause severe damage to the cells of the body. This damage can be to the DNA, proteins and other macromolecules. Oxidative stress, caused by an imbalance between antioxidant systems and the ROS, seems to be associated with many multifactorial diseases, especially cancers, cardiovascular diseases and inflammatory disorders (Reuter et al., 2010; Balmus et al., 2016). The increase in ROS generation or decreased antioxidant availability results in a net increase in intracellular oxidative damage. The mechanism of action of many synthetic antioxidants involves free radical scavenging property, which protects against oxidative damage, but has adverse side effects (Yazdanparast and Ardestani, 2007). The synthetic antioxidant may cause cellular toxicity; however, the alternative is the consumption of natural antioxidants from various food

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supplements and traditional medicines (Yazdanparast et al., 2008).

Terminalia bellerica Roxb (Combretaceae), is a large deciduous tree found throughout India. It is an integral part of ancient formulation, *Triphala* which is used for a variety of ailments. It has been valued in Ayurvedic and traditional system of medicine because of its many pharmacological properties such as anti-inflammatory, immunomodulatory, anticancer, hepatoprotective and antioxidant activities (Rashed et al., 2014; Saraphanchotiwitthaya and Ingkaninan, 2014; Ghate et al., 2014; Gupta et al., 2015; 2016). Gas chromatography mass spectrometry (GC-MS) analysis revealed that *T. bellerica* contains various polyphenolic and other bioactive compounds such as gallic acid, quinic acid (chlorogenic acid), ethyl galate, 9,12 octadecadienoic acid, glucopyranose, sitosterol, protein, tannins, galactose, glucose, mannitol, fructose, ramnose etc. (Gupta et al., 2016). Antioxidant activities increase proportionally with the phenolic content, primarily because of their redox properties (Gupta et al., 2015, 2016). Polyphenolic compounds provide protection against oxidative damage and lower the risk of various oxidative stress associated degenerative diseases by acting as potent free-radical scavengers (Pandey and Rizvi, 2009). This study is focused on the evaluation of the antioxidant, DNA damage protective and antibacterial activities of methanolic and aqueous extracts of *T. bellerica* and correlation of antioxidant studies with total phenolic content (TPC).

MATERIALS AND METHODS

Plant materials

Plant materials were collected (November, 2014) from herbal garden of Narendra Dev University of Agriculture and Technology Kumarganj, Faizabad, India and identified at the Botany Division, CSIR-Central Drug Research Institute, Lucknow, India and the voucher specimens (2322 CSIR-CDRI) were submitted in CDRI herbarium.

Chemicals and reagents

Quercetin, gallic acid, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and thiobarbituric acid (TBA), ethidium bromide was purchased from Sigma-Aldrich, St. Louis, USA. Ascorbic acid, Folin Ciocalteau's phenol reagents were the product of E. Merck, Mumbai, India. Nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), reduced nicotinamide adenine dinucleotide (NADH), potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride ($FeCl_3$), ferrous sulphate ($FeSO_4$) and sodium dodecyl sulphate (SDS) were purchased from Sisco Research Laboratories (SRL), India. All other reagents and chemicals used were of analytical grade.

Extraction procedure

Twenty grams of dried and powdered plant sample of *T. bellerica* from fruit pulp, seed and bark were extracted using 70% methanol (in distilled water) and water (100% distilled water) for overnight shaking at room temperature. The methanolic fruit pulp (MEFP), seed (MES), bark (MEB) and aqueous fruit pulp (AQFP), seed (AQS) bark (AQB) extracts were separated from the residues by filtering through Whatman No. 1 filter paper. The residues were extracted until decoloration with the same fresh solvent and extracts combined. The combined extracts were concentrated and freed of solvent under reduced pressure at 40°C by using a rotary evaporator and lyophilized till dryness. The dried crude concentrated extracts were stored at -4°C and used for the antioxidant activity determination (Gupta et al., 2015).

Antioxidant studies

Total phenolic content (TPC)

TPC of powdered plant material was measured by the method of Ragazzi and Veronese (1973). The TPC was reported as mg of gallic acid equivalent (GAE)/g of dry weight.

Free radical scavenging activity (FRSA)

FRSA of the extracts was measured using DPPH stable radical according to the method of Yen and Duh (1994). The reduction of the DPPH radical was measured by decrease in absorbance at 515 nm until a stable value was obtained.

Inhibition (%) = [(blank absorbance-sample absorbance)/blank absorbance] × 100

The inhibitory concentration (IC_{50}) which represents the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, representing a parameter widely used to measure the antioxidant activity, was calculated from a calibration curve by linear regression.

Superoxide anion radical scavenging activity (SARSA)

This assay was based on the capacity of the extract to inhibit the reduction of nitro blue tetrazolium (NBT) (Nishikimi et al., 1972). The percentage inhibition (PI) of superoxide ($O_2^{\bullet-}$) generation was measured by comparing the absorbance of the control and those of the reaction mixture containing test sample.

Reducing power (RP)

RP of the extract was determined using method of ferric reducing-antioxidant power assay (Apati et al., 2003). RP was expressed as ascorbic acid equivalents (1 ASE = 1 mM ascorbic acid). ASE value is inversely proportional to RP.

Lipid peroxidation (LPO)

A modified thiobarbituric acid-reactive species (TBARS) assay method of Ohkawa et al. (1979) was applied to measure the LPO formation. The absorbance was measured at 532 nm by UV-Vis spectrophotometer (Labtronics, model LT-2910).

Hydroxyl radical scavenging activity (HRSA)

$OH^{\bullet}$ was measured by the method of Halliwell et al. (1987). The reaction mixtures (50 µM ascorbic acid, 20 µM FeCl3, 2 mM EDTA,

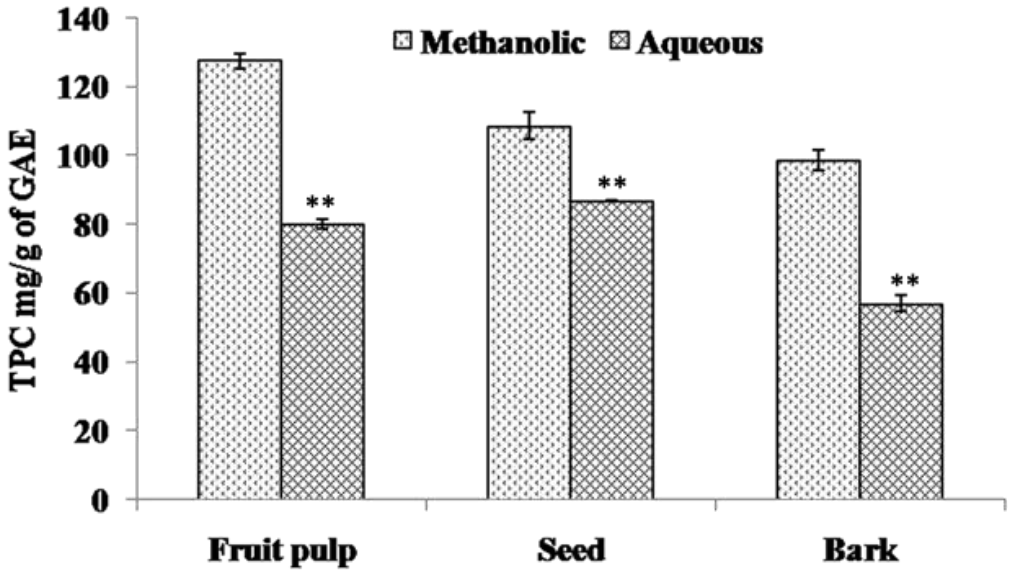

Figure 1. Total phenolic content of methanolic and aqueous extracts of *T. bellerica.* Values are mean±SD of three replications (n=3). MEFP: Methanolic fruit pulp; MES: Methanolic seed; MEB: Methanolic bark; AQFP: Aqueous fruit pulp; AQS: Aqueous seed; AQB: Aqueous bark. **, Results were considered significant at P < 0.01, when compared with methanolic extracts.

1.42 mM H_2O_2 and 2.8 mM deoxyribose) were prepared with different concentrations of the plant extracts. The mixture was heated in a boiling water bath for 30 min. It was cooled and the absorbance was taken at 532 nm.

Ferric thiocyanate assay (FTC)

The reaction mixture (different concentrations of plant extracts, linoleic acid (200 µl) and phosphate buffer 400 µl, pH 7.4) was incubated at 40°C for 15 min. Red color developed was measured at 535 nm (Tsuda et al., 1994).

DNA damage assay

The DNA damage assay was performed using supercoiled pBR322 plasmid DNA by method of Lee et al. (2002).

Antibacterial study

Bacterial culture

Clinical isolates of bacterial strains were obtained from the Department of Microbiology, Dr. Ram Manohar Lohia Avadh University, Faizabad, Uttar Pradesh, India. Bacterial strains were confirmed by using staining and biochemical reactions (Gaur et al., 2015). All the test strains were maintained at 4°C on nutrient agar (Hi-media, Mumbai, India).

Antibacterial activity assay

Broth microdilution method

The broth microdilution method was carried out in a 96-well microtiter plate to determine the minimum inhibitory concentration (MIC). The different concentrations of compounds (200, 150, 100 and 50 µg/ml) were diluted in Mueller Hinton broth and the final volume was maintained upto 200 µl. The final concentration of DMSO was less than 1%. 5 µl of an overnight grown bacterial culture was added to the test medium to bring the final inoculum size to 1×10^5 cfu/ml (Kannan et al., 2006). The agar plates were incubated at 37°C for 24 h and the absorbance was read at 600 nm. The percent growth inhibition was calculated by comparison with a control using the formula indicated below. The lowest concentration of the compound that inhibits the complete growth of the bacterium was determined as the MIC.

$$\% \text{ of growth inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Statistical analysis

All analytical data were subjected to an analysis of variance (ANOVA). Each value is mean ± standard deviation (SD) of three replications (n=3). Statistical analysis was conducted using prism software (GraphPad prism software version 3.0, USA). The results obtained were considered statistically significant at *P<0.05 and **P<0.01. The regression equation and R^2 value were calculated by plotting a graph showing the TPC on the x axis and the antioxidant deciding parameters on the y axis, using MS office excel 2007.

RESULTS AND DISCUSSION

Total phenolic content (TPC)

The TPC in methanolic and aqueous extracts of *T. bellerica were* in the range of 98.68 to 127.60 mg/g GAE and 56.97 to 80.11 mg/g GAE (Figure 1). The highest value of TPC was present in MEFP (127.60 mg/g GAE)

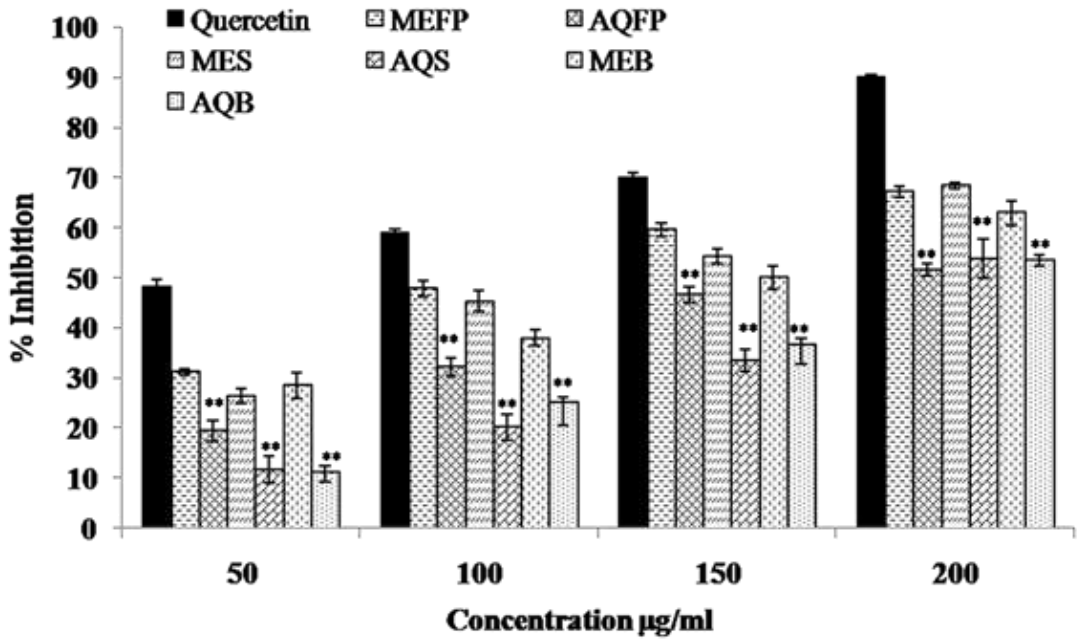

Figure 2. Free radical scavenging activity of methanolic and aqueous extracts of *T. bellerica* and standard quercetin against DPPH radicals at varying concentrations. Values are mean ± SD of three replications (n=3). MEFP: Methanolic fruit pulp; MES: Methanolic seed; MEB: Methanolic bark; AQFP: Aqueous fruit pulp; AQS: Aqueous seed; AQB: Aqueous bark. **, Results were considered significant at P < 0.01 when compared with methanolic extracts.

followed by MES (108.58 mg/g GAE) and MEB (98.68 mg/g GAE) which signifies its high antioxidant activity than aqueous extracts.

Phenols and polyphenols are the most abundant chemical constituents in plants. The antioxidant properties of phenolic compounds originate from their properties of donating e^- to free radicals to stabilize them. Therefore, the quantity of TPC in methanolic and aqueous extracts of *T. bellerica* was determined (Figure 1) in order to determine the antioxidant capacity of plant extracts (Jang et al., 2010; Sampath Kumara et al., 2012). According to Shahriar et al. (2012) and Venkatesan et al. (2017) hexane and chloroform extracts of *Terminalia arjuna* fruit (73.00 and 61.72 mg/g of GAE) and *Terminalia chebula* bark (28.68 and 62.68 mg/g of GAE) exhibited lower TPC in comparison to the reported values of methanolic extracts in this study.

Free radical scavenging activity (FRSA)

Methanolic and aqueous extracts of *T. bellerica* were examined for their potential to scavenge free radicals and measured as percentage inhibition (PI). Methanolic extracts were considered to be most potent free radical scavenger than aqueous extracts and its value of inhibition ranged from 62.95 to 68.34% (Figure 2). FRSA values of methanolic extracts were in the following order MES (68.34%) > MEFP (68.34%) > MEB (62.95%) while for the aqueous extracts the order was AQS (53.82%) > AQB (53.50) > AQFP (51.58%). The IC_{50} value of MEFP was 118.7 µg/ml whereas, in the case of MES and MEB, it was 130 and 146.7 µg/ml, respectively.

The $DPPH^{\bullet}$, at its maximum wavelength at 517 nm, can easily receive an electron or hydrogen from antioxidant molecules to become a stable diamagnetic molecule as DPPH-H (Soares et al., 1997). Owing to the $DPPH^{\bullet}$ ability to bind H, it is considered to have a radical scavenging property. Discoloration occurs due to the decreasing quantity of $DPPH^{\bullet}$ into reaction mixture which reflects the FRSA of tested extract (Guo et al., 2007). The radical scavenging activity of the methanolic extract was found significantly higher in comparison to the aqueous extract.

Superoxide anion radical scavenging activity (SARSA)

The antioxidant potential of methanolic extracts against $O_2^{\bullet-}$ was considered to be significant in comparison to the standard quercetin. The result presented in Figure 3 shows that methanolic extracts inhibited NBT reduction higher than aqueous extracts. The order of SARSA of methanolic and aqueous extracts were MEFP (75.86%) > MEB (73.86%) > MES (72.99%) and AQFP (62.91%) > AQS (56.82%) > AQB (56.19) in comparison to standard quercetin (91.77%) at 200 µg/ml concentration. The IC_{50} values at which methanolic extracts showed significant

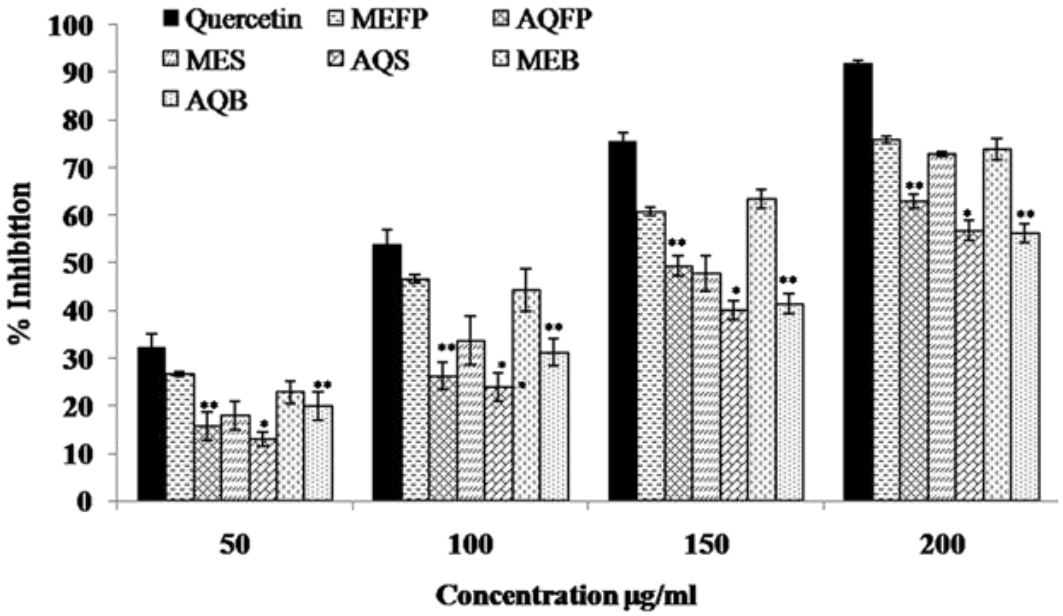

Figure 3. Inhibitory effects of methanolic and aqueous extracts of *T. bellerica* and standard quercetin on superoxide anion radical at varying concentrations. Values are mean±SD of three replicates (n=3). MEFP: Methanolic fruit pulp; MES: Methanolic seed; MEB: Methanolic bark; AQFP: Aqueous fruit pulp; AQS: Aqueous seed; AQB: Aqueous bark. *,**, Results were considered significant at $P < 0.05$ and $P < 0.01$, respectively when compared with methanolic extracts.

SARSA were found as: 77.65 (MEFP), 155.53 (MEB) and 191.75 (MES) µg/ml.

The $O_2^{\bullet-}$ is a precursor to active free radicals that have the potential of reacting with biological macromolecules and thereby inducing tissue damage (Halliwell and Gutteridge, 1984). It has been implicated in several pathophysiological processes due to its transformation into more ROS such as $OH^{\bullet}$, H_2O_2, $^1O_2$ and oxidizing agents that initiate LPO (Wickens, 2001; Liu et al., 1997) and damage to protein and DNA (Pietta, 2000). $O_2^{\bullet-}$ derived from dissolved oxygen by PMS-NADH coupling reaction and reduces NBT in this system. In this method, $O_2^{\bullet-}$ reduces the yellow dye (NBT^{2+}) to produce the blue formazan which is measured spectrophotometrically at 560 nm. Plant extracts containing antioxidants are able to inhibit the formation of blue tetrazolium complex (Cos et al., 1998; Parejo et al., 2002). The decrease of absorbance at 560 nm with antioxidants indicates the consumption of $O_2^{\bullet-}$ in the reaction mixture. Figure 3 clearly indicates that *T. bellerica* is a potent $O_2^{\bullet-}$ scavenger. According to Venkatesan et al. (2017) methanolic extracts of *T. bellerica* bark showed $O_2^{\bullet-}$ inhibition at IC_{50} 166.29 µg/ml which is almost equal to the study reported value of 155.53 µg/ml.

Lipid peroxidation (LPO)

Studies on the inhibition of LPO in the presence of extracts were carried out and expressed as PI. The methanolic and aqueous extracts prevented LPO induced by $FeSO_4$ and PI varied from 63.02 to 71.51 and 54.40 to 60.75%, respectively. Maximum LPO inhibition was shown by MEFP (71.51%) than MES (64.70%) and MEB (63.02%) in a concentration dependent manner at 100 to 400 µg/ml in comparison to standard (95.39%). Methanolic extracts exhibited anti-LPO activity with lower IC_{50} values 115.6 (MEFP), 130.3 (MES) and 144.5 (MEB) µg/ml compared to aqueous extracts 290 (AQFP), 300 (AQS) and 331 (AQB) µg/ml (Figure 4).

In LPO assay, antioxidant potential is measured by evaluating the capability of the test sample to hamper the oxidation of polyunsaturated fatty acids (PUFA) into TBARS. Peroxidation generates peroxyl radicals which decompose to MDA. It forms a stable product with TBA, which serve as a mean to quantify the level of peroxidation (Lugasi, 1997). This assay is a very useful mean to assess LPO *in vitro* due to its simplicity and reproducibility. According to Sherin et al. (2015) hexane, chloroform and ethyl acetate extracts of *T. bellerica* leaf showed 50% inhibition at concentrations of 0.350, 0.280 and 0.520 mg/ml which was much higher than the reported values for methanolic extracts.

Hydroxyl radical scavenging activity (HRSA)

Methanolic and aqueous extracts were further studied for their ability to chelate iron and/or to scavenge $OH^{\bullet}$ by using deoxyribose degradation assay. The methanolic

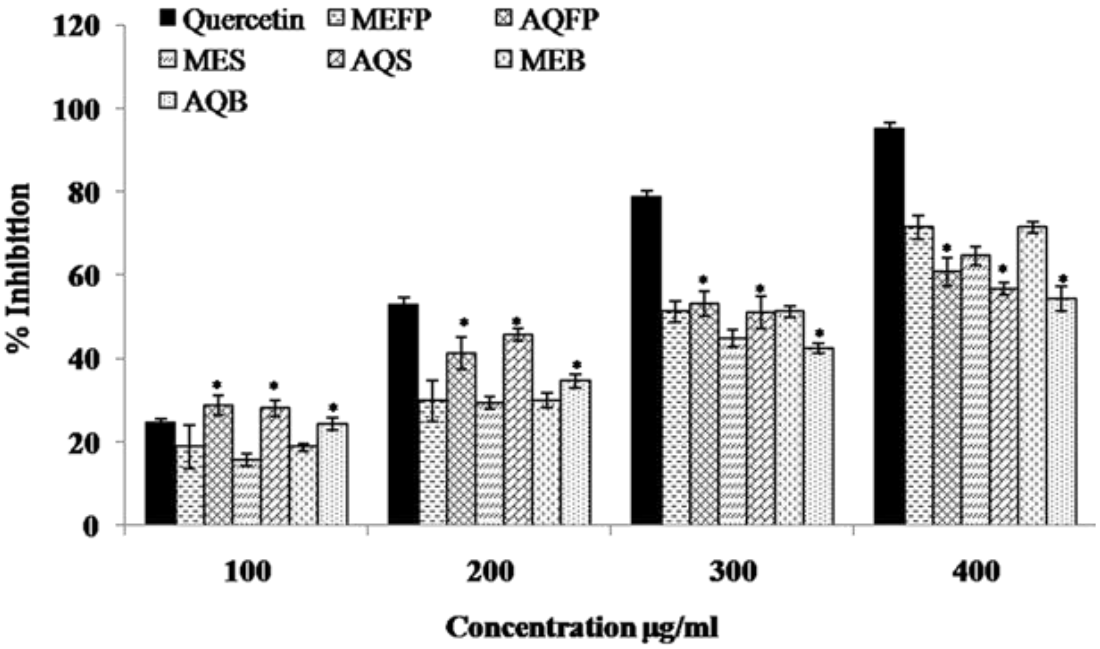

Figure 4. Inhibitory effects of methanolic and aqueous extracts of *T. bellerica* and standard quercetin on LPO using egg homogenate as a lipid-rich source at varying concentrations. Values are mean±SD of three replicates (n=3). MEFP: Methanolic fruit pulp; MES: Methanolic seed; MEB: Methanolic bark; AQFP: Aqueous fruit pulp; AQS: Aqueous seed; AQB: Aqueous bark. **, Results were considered significant at $P < 0.05$ when compared with methanolic extracts.

extracts were found to be most potent $OH^{\bullet}$ scavenger with inhibition of 70.51 to 74.86% than aqueous extracts of 52.70 to 56.23% (Figure 5). The biochemical studies revealed that methanolic extracts caused a concentration-dependent (50 to 200 µg/ml) inhibition of deoxyribose oxidation. The IC_{50} value at which methanolic and aqueous extracts showed HRSA was found to be 73.76 (MEFP), 81.99 (MES), 90.76 (MEB) µg/ml and 425.9 (AQFP), 488.0 (AQS) and 548.0 (AQB) µg/ml, respectively.

The $OH^{\bullet}$ induced oxidative damage to DNA, lipids and proteins are involved in various neurodegenerative and cardiovascular diseases (Spencer et al., 1994). The HRSA of the extracts was determined by its ability to compete with deoxyribose for $OH^{\bullet}$. In this assay, 2-deoxy-2-ribose was oxidized when exposed to $OH^{\bullet}$ generated by the fenton-type reaction. The oxidative degradation can be detected by heating the products with TBA under acidic conditions to develop a pink chromogen with a maximum absorbance at 532 nm (Marnett, 1999).

Ferric thiocyanate (FTC)

In tested extracts, methanolic extracts showed maximum inhibition (76.08 to 79.78%) than aqueous extracts (53.00 to 54.80%) to inhibit the production of free radicals which initiate the oxidation of lipids and proteins. The inhibition was increased with increasing concentration of the extracts from 50 to 200 µg/ml in the reaction mixture and the maximum inhibition was shown by MES (79.78%) in comparison to quercetin (92.12%) (Figure 6). The IC_{50} of methanolic extracts was found to be in the range 184.98 (MEFP) > 212.5 (MES) > 221.4 (MEB).

The antioxidant activity of plant extracts was further determined by the inhibition of peroxidation of linoleic acid system using thiocyanate method (Yen et al., 2000). Linoleic acid is a PUFA which upon oxidation forms peroxides that oxidize Fe^{2+} to Fe^{3+}. Fe^{3+} forms complex with thiocyanate ion (SCN^-), whose concentration is determined spectrophotometrically by measuring the absorbance at 535 nm. Higher absorbance denotes higher concentration of peroxides formed during reaction; consequently lower will the antioxidant activity. According to Sultana et al. (2007) methanolic extracts of *T. arjuna* bark exhibited 44.4% inhibition at 200 µg/ml concentration which was found much lower than the bark extracts tested in the study.

Reducing power (RP)

The RP of a compound may act as a significant indicator of its potential antioxidant activity. With regards to RP, higher reducing capacity might be attributed to the higher amount of phenolic compounds. Methanolic extracts exhibited significantly high Fe^{3+} to Fe^{2+} transformation capacity (6.73 to 4.68 ASE/ml) compared to aqueous

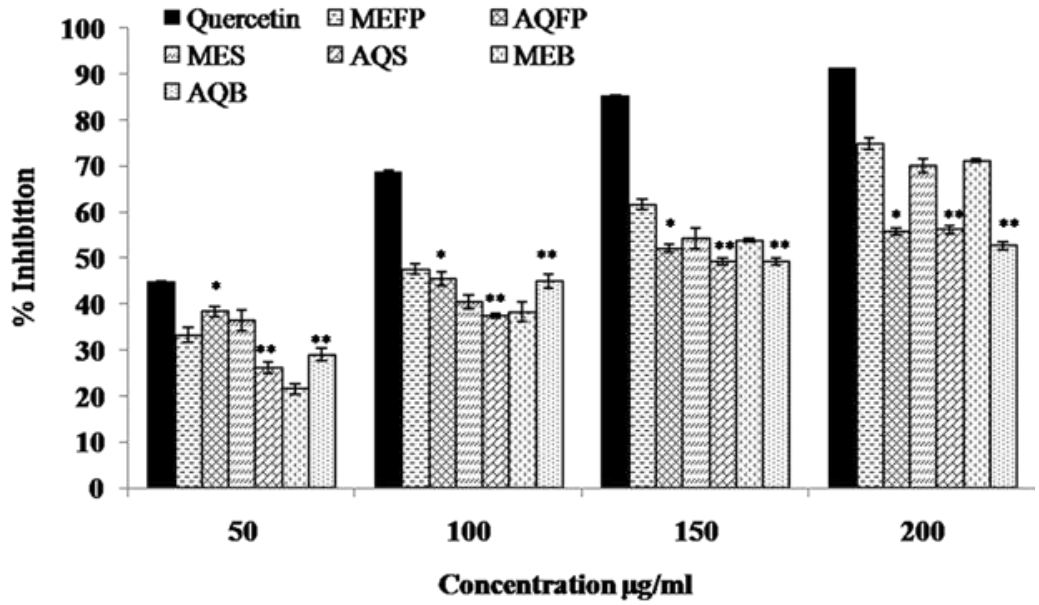

Figure 5. Inhibitory effects of methanolic and aqueous extracts of *T. bellerica* and standard quercetin on hydroxyl radical mediated deoxyribose degradation at varying concentrations. Values are mean ± SD of three replicates (n=3). MEFP: Methanolic fruit pulp; MES: Methanolic seed; MEB: Methanolic bark; AQFP: Aqueous fruit pulp; AQS: Aqueous seed; AQB: Aqueous bark. *,**, Results were considered significant at $P < 0.05$ and $P < 0.01$, respectively when compared with methanolic extracts.

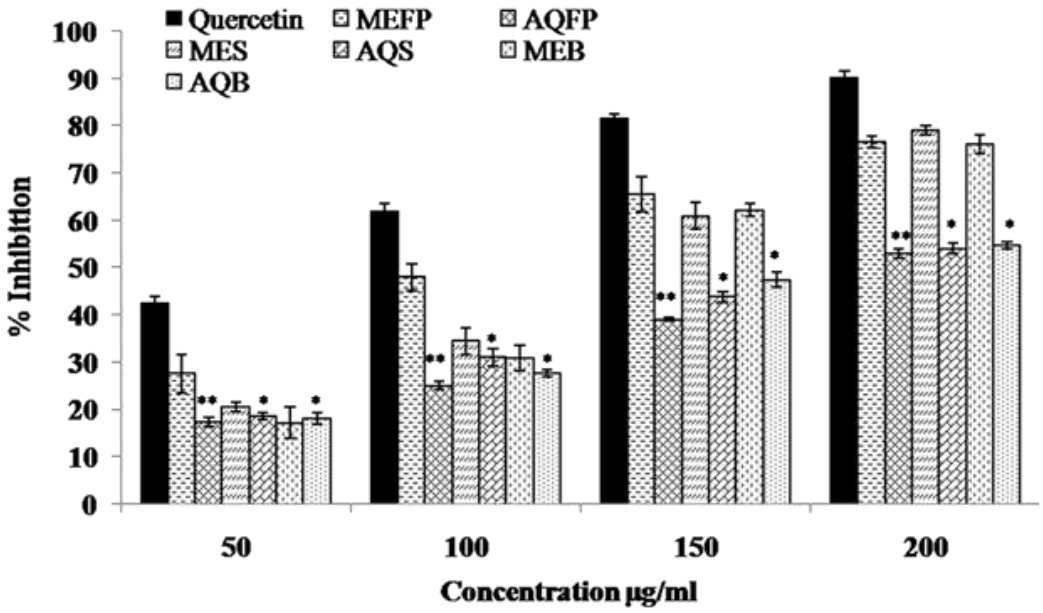

Figure 6. Inhibitory effects of methanolic and aqueous extract of *T. bellerica* and standard quercetin on ferric ion chelation by ferric thiocyanate assay method at varying concentrations. Values are mean ± SD of three replicates (n=3). MEFP: Methanolic fruit pulp; MES: Methanolic seed; MEB: Methanolic bark; AQFP: Aqueous fruit pulp; AQS: Aqueous seed; AQB: Aqueous bark. *,**, Results were considered significant at $P < 0.05$ and $P < 0.01$, respectively when compared with methanolic extracts.

extracts (10.03 to 7.25 ASE/ml (Figure 7). In the RP assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{3+} reduction can be then monitored by measuring the formation of $(Fe^{3+})_4[Fe^{2+}(CN)_6]_3$ complex (pearl's Prussian blue) at 700 nm, indicates an

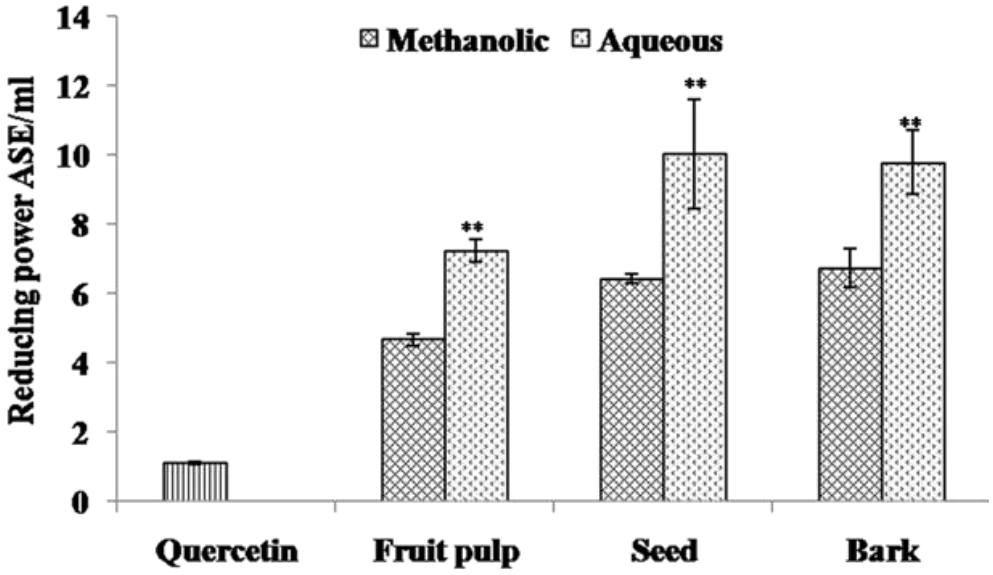

Figure 7. Reducing power (ASE/ml) of methanolic and aqueous extracts of *T. bellerica* and standard quercetin. Values are mean ± SD of three replicates (n=3). MEFP: Methanolic fruit pulp; MES: Methanolic seed; MEB: Methanolic bark; AQFP: Aqueous fruit pulp; AQS: Aqueous seed; AQB: Aqueous bark. **Results were considered significant at $P < 0.01$ when compared with methanolic extracts.

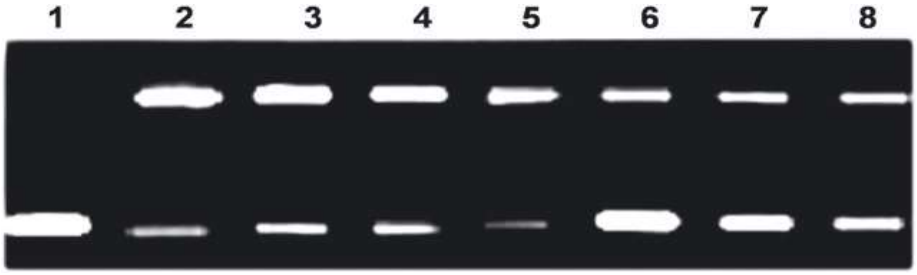

Figure 8. DNA damage protection offered by methanolic (ME) and aqueous (AQ) extracts of *T. bellerica* fruit pulp, seed and bark on native pBR322 DNA nicking caused by hydroxyl radicals. Lane 1: DNA (pBR322 plasmid DNA alone), Lane 2: DNA + Fenton reagent, Lane 3: DNA + Fenton reagent + 50 µg/ml AQFP, Lane 4: DNA + Fenton reagent + 50 µg/ml AQS, Lane 5: DNA + Fenton reagent + 50 µg/ml AQB, Lane 6: DNA + Fenton reagent + 50 µg/ml MEFP, Lane 7: DNA + Fenton reagent + 50 µg/ml MES, Lane 8: DNA + Fenton reagent + 50 µg/ml MEB.

increase in reductive ability (Djeridane et al., 2006). Fe^{3+} reduction is often used as a significant indicator of electron donating activity which is an important mechanism of phenolic antioxidant action (Nabavi et al., 2009).

DNA damage protective activity

Hydroxyl radical scavenging activity of *T. bellerica* extracts was further explored by the protection of plasmid pBR322 DNA against Fenton reagent. When DNA is exposed to Fenton reaction, H_2O_2 will be generated to hydroxyl radicals ($OH^{\bullet}$), and then the supercoiled form of DNA would cleave to give rise to a linear form. The DNA damage protective activity of the plant extracts showed a significant protection of supercoiled form compared to open circular form, at 50 µg/ml. Addition of Fenton reagent to a mixture containing DNA and *T. bellerica* extracts showed a significant reduction in the formation of open circular and linear forms and increased supercoiled or native forms of plasmid DNA as shown in Figure 8.

$OH^{\bullet}$ can be formed by the Fenton reaction, capable of damaging DNA and protein in living cells (Rollet-Labelle et al., 1998). In the present experiment, plasmid pBR322 DNA was treated with Fenton reagent. It interacts with the supercoiled DNA and cleaved into open and nicked circular forms. The results were obtained through gel electrophoresis (Figure 8) showed that the methanolic

Table 1. Minimum inhibitory concentration (MIC) (µg/ml) of methanolic and aqueous extracts of *Terminalia bellerica.*

Bacteria	Extract (µg/ml)	Plant extract							
		MEFP	MES	MEB	AQFP	AQS	AQB	CN (30 µg/disc)	TE(30 µg/disc)
Gram (+)									
Sa	200	19±0.5	19±0.1	21±0.1	19±0.2	16±0.5	20±0.5	15±0.5	17±0.3
	150	22±0.4	23±0.6	28±0.2	23±0.3	20±0.2	24±0.3	21±0.3	22±0.4
	100	29±0.3	28±0.5	34±0.4	31±0.4	25±0.3	29±0.5	26±0.2	27±0.5
	50	34±0.2	33±0.5	39±0.2	35±0.3	31±0.2	33±0.5	31±0.2	34±0.5
Se	200	09±0.3	16±0.3	14±0.3	15±0.3	14±0.1	17±0.1	12±0.2	12±0.1
	150	12±0.5	28±0.2	25±0.4	23±0.0	20±0.2	24±0.2	17±0.3	17±0.3
	100	15±0.1	33±0.1	30±0.1	30±0.1	27±0.5	29±0.5	20±0.4	24±0.4
	50	30±0.5	38±0.1	35±0.1	36±0.0	33±0.1	34±0.3	24±0.4	27±0.4
Bc	200	36±0.1	54±0.1	73±0.1	76±0.1	28±0.1	38±0.1	23±0.4	22±0.3
	150	49±0.1	61±0.1	89±0.1	82±0.1	45±0.2	47±0.2	28±0.2	26±0.4
	100	78±0.5	109±0.2	108±0.1	98±0.0	57±0.5	67±0.5	33±0.3	31±0.2
	50	94±0.5	124±0.2	128±0.2	119±0.0	76±0.1	86±0.1	38±0.3	36±0.2
Gram (-)									
Pa	200	31±0.1	37±0.1	39±0.1	31±0.1	34±0.1	34±0.1	25±0.5	28±0.4
	150	35±0.1	45±0.1	56±0.1	48±0.1	50±0.2	48±0.2	33±0.3	36±0.3
	100	52±0.5	63±0.5	71±0.0	67±0.0	63±0.5	65±0.5	39±0.5	41±0.3
	50	74±0.1	85±0.1	88±0.1	89±0.1	82±0.1	82±0.1	48±0.5	53±0.2
Ec	200	08±0.1	09±0.1	10±0.1	11±0.1	10±0.1	11±0.1	08±0.3	08±0.5
	150	17±0.1	19±0.1	20±0.1	19±0.1	18±0.2	20±0.2	12±0.3	15±0.3
	100	23±0.5	29±0.1	28±0.0	24±0.0	25±0.5	28±0.5	19±0.2	21±0.4
	50	29±0.5	36±0.2	39±0.0	37±0.0	34±0.1	38±0.1	28±0.2	28±0.4

Values are mean ± SD of three replicates (n=3). CN: Gentamicin; TE: Tetracycline; MEFP: Methanolic fruit pulp, MES: Methanolic seed; MEB: Methanolic bark; AQFP: Aqueous fruit pulp, AQS: Aqueous seed; AQB: Aqueous bark; Sa: *S. aureus*; Se: *S. epidermidis*; Bc: *B. cereus*; Pa: *P. aeruginosa*; Ec: *E. coli*

extract of *T. bellerica* significantly protected DNA compared to aqueous extracts. As observed in Figure 8, where lane 1 in gel pictures is the positive control with only plasmid (pBR322) DNA in the fully supercoiled form and lane 2 is a negative control containing Fenton's reagent without plant extract and having only the open circular form. The lanes 3 to 8 in the corresponding gel picture signify DNA protective activity with different extracts of 50 µg/ml. Thus, results suggested that methanolic extracts of *T. bellerica* was better than aqueous extracts in retaining the supercoiled form of DNA, thus protecting DNA effectively against oxidative damage. The result was significant in comparison to earlier reported study by Singh et al. (2016).

Antibacterial activity

Minimum inhibitory concentration (MIC)

MIC is defined as a highest dilution or lowest concentration of a chemical that inhibits the growth of bacteria and thereby estimates its antimicrobial activity. The methanolic and aqueous extract of *T. bellerica* was further subjected to the broth microdilution method to determine the MIC (Table 1). The lowest MIC was observed against *S. aureus, S. epidermidis* and *E. coli* at a concentration of 200 µg/ml. As antimicrobial concentration increases, the turbidity decreases until the MIC is reached and microbes no longer survive. Antimicrobials with low MICs are more effective than those with high MICs, as only a low dosage is necessary to eradicate microbes. This result is in agreement with the report of Kannan et al. (2009) and Bag et al. (2012) studied on *T. chebula* fruits extracts, respectively. According to Bag et al. (2012), *T. chebula* aqueous extracts showed MIC 0.866 mg/ml and 1.65 mg/ml against *E. coli* and *S. aureus* which were much higher than that reported in the tested extracts.

Correlation between antioxidant activities and TPC

Phenolics are the major contributors to the antioxidant activity. The correlation between TPC and FRSA of methanolic and aqueous extracts had a correlation

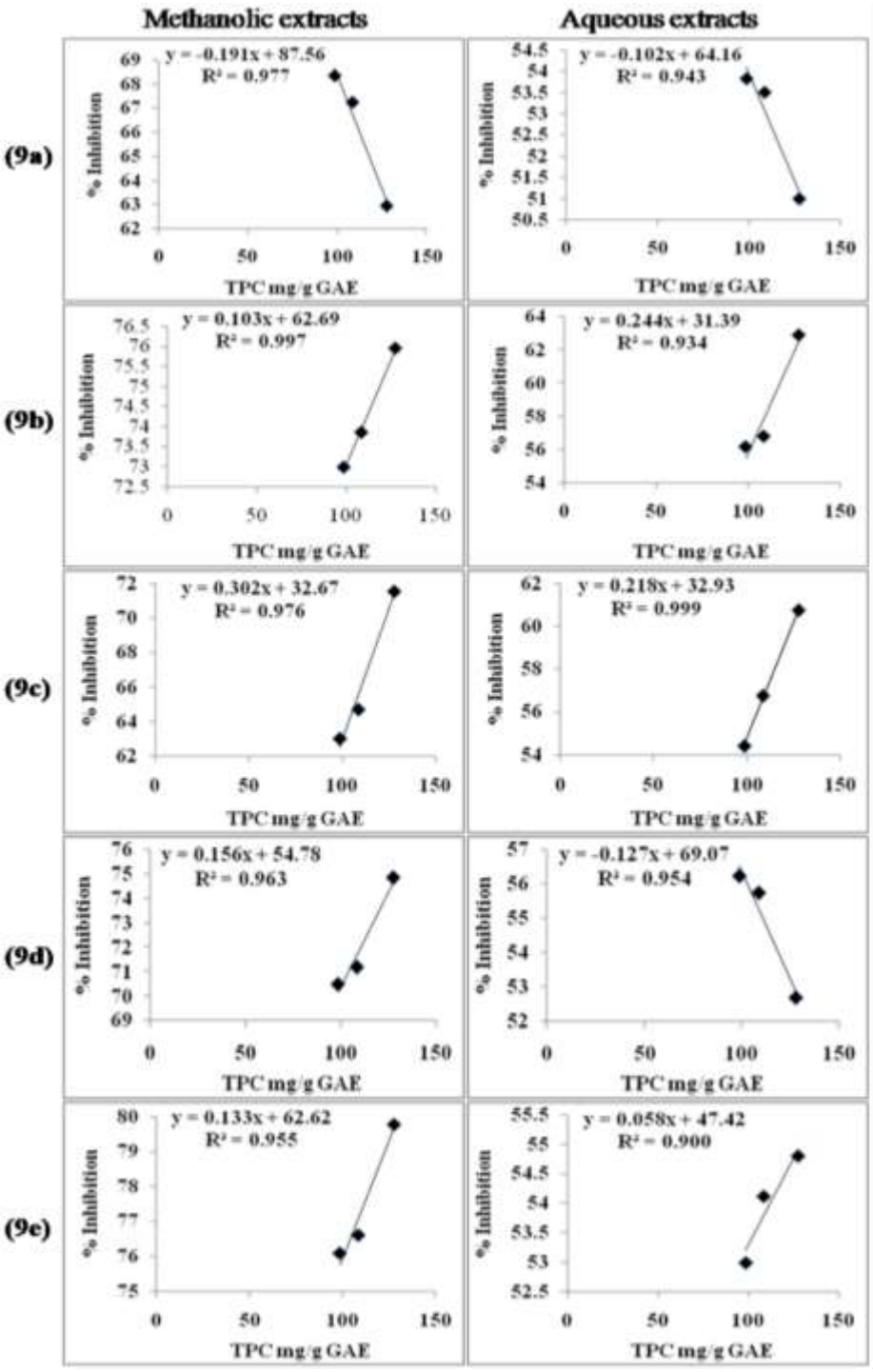

Figure 9. Linear correlation between TPC (x axis) in the plant extracts in relation to their antioxidant activity (y axis). (9a) TPC versus FRSA; (9b) TPC versus SARSA; (9c) TPC versus LPO; (9d) TPC versus HRSA; (9e) TPC versus FTC assay.

coefficient of R^2 = 0.977 (y = -0.191x + 87.56) and R^2 = 0.943 (y = -0.102x + 64.16). A good correlation also exists between TPC and other antioxidant activities (Figure 9).

It was observed that the FRSA of the methanolic and aqueous extracts was positively correlated with their total amount of phenolic compounds (R^2 = 0.977 and 0.943). Many studies in the literature present positive correlations

between the quantity of phenolic compounds and the DPPH$^{\bullet}$ scavenging effect (Sagar and Singh, 2011; Liu et al., 2009). In this study as well, a positive correlation was observed between the antioxidant activities and the content of total phenolics.

Conclusion

This study revealed that *T. bellerica* possesses potent free-radical scavenging activities. Overall, methanolic extracts were found to be a good free-radical scavenger and showed high antioxidative, DNA protective and antibacterial activities compared to aqueous extracts. The resulting activities may be attributed to the presence of more phenolic compounds in the methanolic extracts compared to aqueous extracts. Furthermore, it is concluded that *T. bellerica* is a potential candidate for natural antioxidants in food and pharmaceutical industries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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ABBREVIATIONS

ROS, Reactive oxygen species; **PI,** percentage inhibition; **LPO,** lipid peroxidation; **TBARS,** Thiobarbituric acid-reactive species; **MDA,** Malondialdehyde; IC_{50}, inhibitory concentration; $\mathbf{O_2^{\bullet-}}$**,** superoxide; $\mathbf{OH^{\bullet}}$**,** hydroxyl radical; **PUFA,** polyunsaturated fatty acids.

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Therapeutic role of chitosan nanoparticles in murine schistosomiasis mansoni

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This research evaluates the value of loading *Nigella sativa* on chitosan nanoparticles (ChNPs) in the treatment of *Schistosoma mansoni* infection compared to praziquantel. Chitosan has efficient oral administrative capability to penetrate mucosal surface being mucoadhesive and non-toxic, as well as combination with NPs add characters of controlled release and safe serum levels. The study was done on 40 *S. mansoni* infected male mice which were divided into 4 groups: positive control group (infected, non treated), group treated with *N. sativa* only (NS), group treated with *N. sativa* loaded chitosan NPs (NC) and group treated with combined praziquantel and *N. sativa* loaded chitosan nanoparticles (NCP). Results showed decreased worm burden in NC and NCP groups concerning the control and NS groups while significant decrease occurred in oogram pattern and tissue egg loads reached reduction percentages more than 90% in NC and NCP groups. On the level of granuloma diameter reduction, it was 46.3% in NC and 41.3% in NCP group while granuloma number was reduced by 50.9% in NC and reduced by 32% in NCP group revealing the apparent role of ChNPs in improvement of bilharzial hepatic changes. Thus, ChNPs improved the effects of *N. sativa* in therapy of murine schistosomal infection by enhancing the effects of praziquantel and reducing the resistance to it. This can be considered as a new strategy using ChNPs as anti-schistosomal drug carrier in mice models.

Key words: *Nigella sativa,* chitosan nanoparticles, *Schistosoma mansoni,* praziquantel

INTRODUCTION

Regardless of the many efforts to control schistosomiasis infection in Egypt, it is still one of the most common endemic diseases achieving high prevalence in Egypt like many other developing countries. Schistosomiasis represents one of the most common parasitic diseases worldwide and the second most harmful parasitic infection after malaria in the aspect of economic impact and disabling infection (Ali et al., 2016).

Drug-resistant *Schistosoma* strains were shown in endemic areas due to the repeated use of praziquantel

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and oxamniquine (Zhang and Coultas, 2013), hence in the last few years, there was an increased request for the use of anti-parasitic drugs of natural plant sources (Magalhães et al., 2009). Accordingly, the importance of exploring new effective natural compounds to be used in treatment of schistosomiasis is a must (John et al., 2007).

Nigella sativa oil is considered one of the plant derivatives which have been screened as active being active agent for adult *Schistosoma mansoni* and is a promising drug of a plant origin that has anti-schistosomal efficacy (Mohamed et al., 2005).

Seeds of *N. sativa* which are known as black seeds were a fertile source for treatment of abdominal pain, diarrhea, cough, asthma, and rheumatism and other disease as a part of traditional medicine in Asia, Middle and Far Eastern countries. Many phytochemicals and pharmacological studies proved the antioxidant, anti-inflammatory, anticancer and antimicrobial activities of the aqueous and oil extracts of these seeds. The incorporation of *N. sativa* into solid nanoparticles as a new delivery system revealed them as a suitable carrier in the pharmaceutical fields with high physical stability (Al-Haj et al., 2010).

Chitosan is produced by removal of acetate moiety of chitin, a powerful source of chitosan is the crustacean cells of crabs in addition to fungal cell walls, and chitosan is a natural biocompatible polymer, which is characterized by being highly basic, mucoadhesive, and cationic polysaccharide. Being a penetration enhancer, chitosan promotes both paracellular and intercellular transport of drugs by opening the tight junctions of the epithelium (Mohammed et al., 2017).

On testing resistance of chitosan NPs to the effect of pH and temperature in media that simulate the stomach and intestine, it was found very stable at 37°C in different buffers (Oliveira et al., 2012). Being a polysaccharide source for nanoparticles, chitosan was successfully applied as drug delivery system because polysaccharides are stable, safe, non-toxic, hydrophilic and biodegradable; also, the electrostatic interaction between positively charged polymers as chitosan and negatively charged mucin makes chitosan more mucoadhesive, as well as its initial burst effect and slow drug release which were proven *in vitro* contribute to their urgent necessity to be used *in vivo* ChNPs (Bilia et al., 2014).

Nanoparticle technology became one method offering a valuable tool to novel drug delivery strategies, having the characters of controlled release, safe serum levels of active components from enzymatic or environmental degradation and internal retention. Nanoparticle manufacturing methods apply to a wide range of drugs (Nagpal et al., 2010). Chitosan-based nanoparticles are also used in the treatment of cancer, gastrointestinal diseases, pulmonary diseases, drug delivery to the brain and ocular infections (Mohammed et al., 2017). The aim of this study is to evaluate the value of loading *N. sativa* on chitosan nanoparticles (ChNPs) in treatment of *S. mansoni* adult worms infection compared to praziquantel.

MATERIALS AND METHODS

This experimental study was conducted in the Schistosoma Biological Supply Centre (SBSC), Theodore Bilharz Research Institute of Giza in Egypt during the period from July 2018 to October 2018. No specific grant from any funding agency was provided towards this research.

Materials and drugs

Chitosan (deacetylation degree of 93%) and sodium tripolyphosphate (Na TPP) were purchased from *Sigma-Aldrich, USA*. PBS and acetic acid were obtained from *Sigma–Aldrich, USA*. *N. sativa* oil (Baraka) in the form of gelatinous capsules 450 mg was obtained from *Pharco, Egypt*. The inotropic gelation of chitosan with Na TPP anions helps in the synthesis of ChNPs (Kawashima et al., 1985; Werle et al., 2009). This interaction was controlled by the charge density of Na TPP and chitosan under the effect of the solution pH. Various concentrations of acetic aqueous solutions 1, 2 and 3 mg/ml were used to dissolve chitosan. Na TPP solution (1 mg/ml) was prepared by double-distilled water. ChNPs were produced by dropwise addition of 5 ml of the chitosan solution on 2 ml of Na TPP solution by the effect of 1000 rpm magnetic stirring for 1 h at room temperature. By centrifugation at 20000 g at 14°C for 30 min, we did separations of the nanoparticles and then they were freeze-dried and stored at 4°C.

Loading of *N. sativa* to ChNPs was made by adding chitosan solution to Na TPP solution (containing *N. sativa* at a concentration of 500 mg/2 ml). *N. sativa* loaded on ChNPs (NS/ChNPs) was separated from the suspension by centrifugation (20000 g at 14°C) for 30 min. Thereafter, sediment was collected and weighed. The particle size was determined by dynamic light scattering (DLS) using Zetasizer Nano Instrument (*Malvern Instruments, UK*) according to (Koukaras et al., 2012) to record that at chitosan/Na TPP w/w ratio of 2.5/1, the nanoparticle diameter was 200-396 nm and physical appearance was opalescent suspension. The total protein content/mg of chitosan encapsulating powder was calculated by dividing the protein concentration of the loaded *N. sativa*/the nanoparticles' weight (Danesh-Bahreini et al., 2011). The loading efficiency of the nanoparticles was determined by:

$$\%LC = [(A-B)/C] \times 100$$

Where A letter points to the total amount of *N. sativa*, B letter points to the free amount of *N. sativa* and C letter points to the weight of nanoparticles.

N. sativa (Baraka) gelatinous capsules (450 mg) were obtained from *Pharco Pharmaceuticals, Egypt,* dissolved in corn oil and adjusted to be given at dose 1140 mg/kg. Praziquantel tablets (Distocide, EIPICO, El-Asher Men Ramadan, Egypt) were crushed, administered orally as a suspension in 2% Cremophore-E1 (Sigma-Aldrich Chemical Co., St. Louis, MO) (Fallon and Doenhoff, 1994). Praziquantel was given to mice in a dose of 500 mg/kg divided in half and given on two consecutive days (half full dose).

Experimental design

40 male mice, aged 6-8 weeks and weighed 20–25 g, were included in our study. Mice were housed in well-ventilated cages and fed standard pellet food with free access to water (El Fakhry et al., 1998). Mice were divided into four groups (10 mice/group):

Control group: control positive, infected mice and not treated.
NS group: treated with *N. sativa* only.
NC group: treated with NS/ChNPs.
NCP group: treated with combined praziquantel and NS/ChNPs.

Mice infection and treatment

Cercariae of *S. mansoni* were obtained from infected *Biomphalaria Alexandrina* snails, reared and maintained at Schistosome Biological Supply Program (SBSP), Theodore Bilharz Research Institute, in the governorate of Giza, Egypt. Mice were infected subcutaneously with freshly shed 60±10 cercariae/mouse (Liang et al., 1987). The treatment schedule included that *N. sativa* only was given by a dose of 1140 mg/kg day after day. NS/ChNPs was given in a dose of 1140 mg/kg daily. Combined praziquantel and NS/ChNPs were given at a dose of 500 mg/kg praziquantel divided in half and given on two consecutive days + 570 mg/kg NS/ChNPs daily. Treatment started from the 7^{th} week post-infection and continued for two weeks. All mice were sacrificed by cervical dislocation at 9 weeks post-infection.

Worm recovery

The worm burden of *S. mansoni* recovered from the hepatic portal system and mesenteric veins of sacrificed mice was done by the perfusion technique described by (Smithers and Terry, 1965).

Egg count examination

a) Oogram pattern: small intestine of sacrificed mice was divided into 3 parts (each 1 cm in length), then squeezed between a glass slide and coverslip to show and count immature, mature and dead egg stages of *S. mansoni* in each part and the mean number of each stage was calculated (Pellegrino et al., 1962).
b) Tissue egg load: the number of ova per gram the liver or intestinal tissue was determined by digesting a part of liver or middle intestine in 5% potassium hydroxide overnight (Cheever, 1968).

Histopathological examination

It is done by fixing liver samples from all groups of the experiment in a 10% formalin solution and then embedding these samples in paraffin wax and staining of five sections with hematoxylin and eosin (Bancroft and Stevens, 1975) and Masson Trichrome stain (Masson, 1929). The number of liver egg granulomas was assessed by microscopic examination of the prepared slides through five successive low power fields (10X) while the granuloma mean diameter was calculated by dividing the sum of vertical and transverse diameters/2 (Mahmoud and Warren, 1974).

Ethical considerations

The Ethical Committee of the Faculty of Medicine, Benha University, Egypt and that of Theodore Bilharz Research Institute approved the study according to the international guiding principles for biomedical research involving animals as issued by the international organizations of medical sciences.

Statistical analysis

Results were collected, tabulated and statistically analyzed using the statistical package SPSS version 12. Data were tabulated as mean and standard deviation (SD) for quantitative variables and percent for qualitative variables. ANOVA was used to detect significance in the quantitative variables, and P values <0.05 were considered as statistically significant. Post-hoc Bonferroni test was used.

RESULTS

Parasitological study

Worm burden

The mean female worm burden of control mice and NS group was 3.4±0.51 and 0.6±0.96 respectively with no female worms found in NC and NCP groups achieving reduction percentage of 100% while the mean male worm burden in control, NS and NC groups was 0.9±0.87, 0.4±1.07 and 0.3±0.48 respectively with no male worms in NCP group. Also, mean couple worm burden in control, NS and NC groups was 5±1.15, 1±0 and 0.6±0.51 respectively showing high reduction percentage of 88% in NC group and no couple worms in NCP group while total worm burden in the same group was 13.8±1.22, 4.65±2.62 and 3.1±0.48 and 0 respectively. These results revealed achievement of a reduction in total worm burdens from the control group by 66.3, 77.5 and 100% in NS, NC and NCP groups respectively (Table 1, Graph 1).

Oogram pattern

Per every 100 eggs in control group mice we found that mean number of immature, mature and dead eggs was 52±2.3, 44.6±1.07 and 3.4±2.5 respectively while in NS group mice the mean number of immature, mature and dead eggs was 14±5.1, 69.5±7.2 and 26.5±6.2 respectively. Regarding the NC group mice the mean number of immature, mature and dead eggs was 4±8.7, 53.5±2.4 and 32.5±6.7 respectively while in NCP group mice, there was no immature eggs; also, mean number of mature and dead eggs was 35.9±2.9 and 64.1±2.9 (Table 2, Graph 2) regarding the mean number of immature eggs.

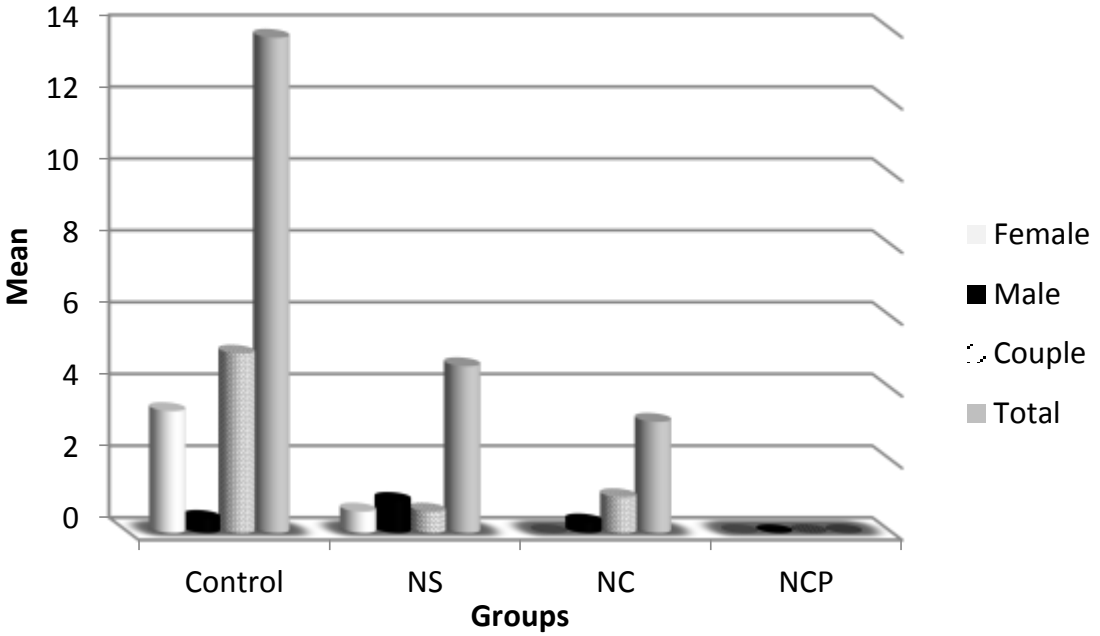
Tissue egg load (No. of eggs per gram)

The mean liver egg count of control, NS, NC and NCP groups of mice was 26851±2341, 5298.55±2949, 4169.38±1264.6 and 2402.54±1127 respectively with highest reduction percentages from control in NC and NCP groups by 84.5 and 91.1% respectively, while the mean intestine egg count of control, NS, NC, NCP groups of mice was 35348±3416, 16098± 3069, 11380±2548 and 3387±597 respectively with highest reduction percentages in NC and NCP groups as 67.8 and 90.4% respectively;

Table 1. Effect of treatment on worm burden in NS, NC and NCP groups compared to control experimental mice.

Group	Worm burden (Reduction %)			
	Female (R %) mean±SD	Male (R %) mean±SD	Couple (R %) mean±SD	Total (R %) mean±SD
Control	3.4±0.52	0.9±1.07	5±1.15	13.8±1.22
NS	0.6±0.96 (R 82.4%)	0.4±0.87 (R 55.5%)	1±0 (R 80%)	4.65±2.62 (R 66.3%)
NC	0 (R 100%)	0.3±0.48 (R 66.6%)	0.6±0.51 (R 88%)	3.1±0.48 (R 77.5%)
NCP	0 (R 100%)	0 (R 100%)	0 (R 100%)	0 (R 100%)

i) P<0.001(highly significant) in comparing the mean total worm burden between NC, NS, NCP groups. And post hoc analysis revealed that the statistically significant difference was between NS, NCP, and NC, NP groups. ii) P<0.001 (highly significant) in comparing mean total worm burden between control and NCP, control and NS, control and NC groups. iii) R = reduction % from control group.

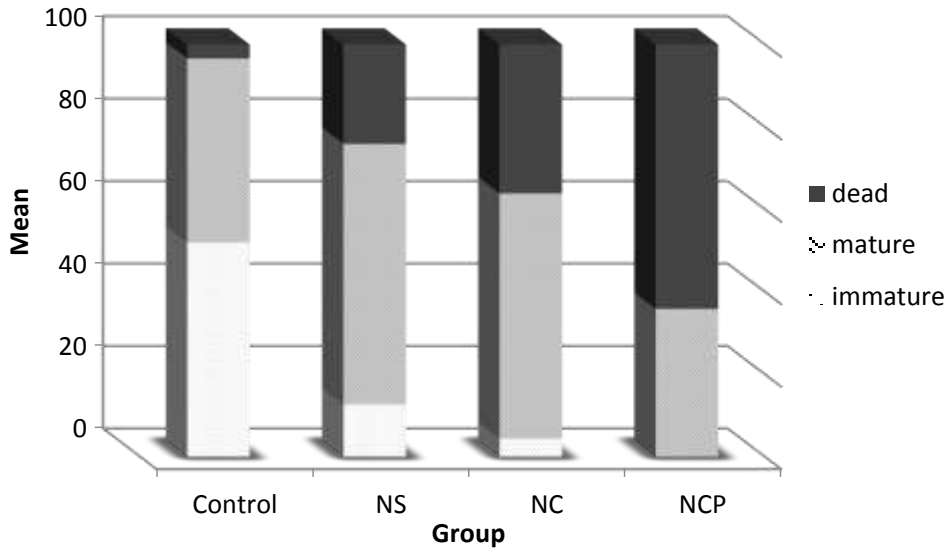


Graph 1. Worm burdens in NS, NC and NCP groups of mice against control group.

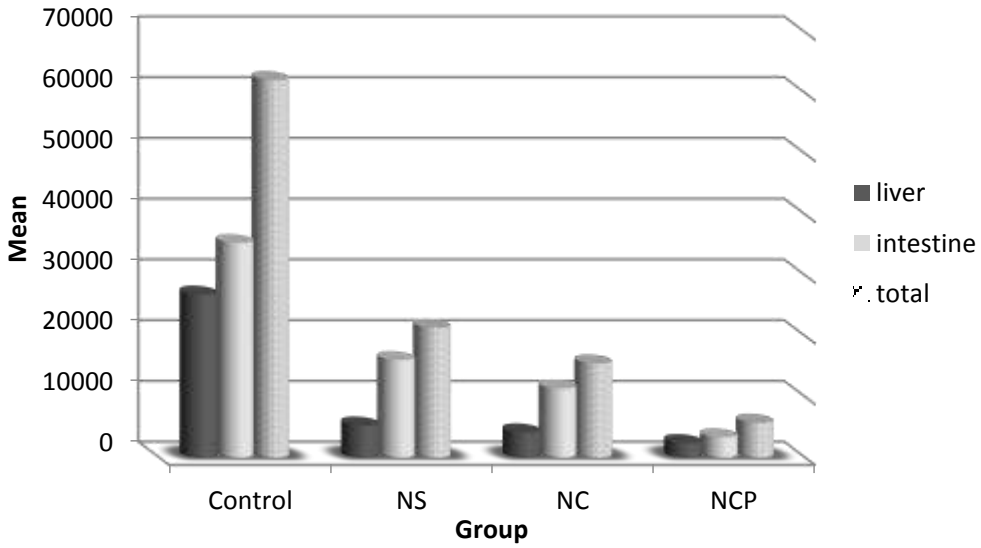
Table 2. Effect of treatment on oogram pattern and tissue egg loads in NS, NC, and NCP groups compared to control experimental mice.

Group	Oogram pattern			Tissue egg load (Reduction %)		
	Immature	Mature	Dead	Liver	Intestine	Total
	Mean±SD			Mean±SD		
Control	52±2.3	44.6±1.07	3.4±2.5	26851±2341	35348±3416	62199 ±5757
NS	14±5.1	69.5±7.2	26.5±6.2	5299± 2949 (R 80.2%)	16098± 3069 (R 54.5%)	21396±5952 (R 65.6%)
NC	4±8.7	53.5±2.4	32.5±6.7	4169±1265 (R 84.5%)	11380±2548 (R 67.8%)	15550±3793 (R 75%)
NCP	0	35.9±2.9	64.1±2.9	2403±1127 (R 91.1%)	3387±597 (R 90.4%)	5789±1724 (R 90.7%)

i) P<0.001 (highly significant) in comparing each of immature, mature and dead eggs between NS, NC, and NCP groups (ANOVA). ii) Regarding the mean value of immature ova, post HOC analysis revealed that the statistically significant difference was between NS, NC, and NS, NCP groups. iii) Regarding the mean value of mature ova, post HOC analysis revealed that the statistically significant difference was between NS, NC and NS, NCP, and NC, NCP groups. iv) Regarding the mean value of dead ova, post hoc analysis revealed that the statistically significant difference was between NS, NCP, and NC, NCP groups. v) P<0.001 (highly significant) in comparing each of immature, mature and dead ova between control and NCP, control and NS, control and NC (t-test). vi) P<0.001(highly significant) in comparing total tissue egg load between NS, NC, and NCP groups. And post hoc analysis revealed that the statistically significant difference was between NS and NCP, NS and NC, NC and NCP groups. vii) P<0.001 (highly significant) in comparing total tissue egg load between control and NCP, control and NS, control and NC groups. viii) R = reduction % from control group.



Graph 2. Oogram pattern in NS, NC and NCP groups of mice against control group.



Graph 3. Tissue egg loads in NS, NC and NCP groups of mice against control group.

also, the mean total tissue egg load for the same groups respectively was 62199±5757, 21396±5952, 15550±3793 and 5789±1724 (Table 2, Graph 3).

Histopathological study

The mean diameter of liver granuloma of control, NS, NC, and NCP groups was 187.86±31.5, 143.20±35.5, 100.74±25.1 and 110.12±24 with reduction of the NS, NC and NCP groups from control by 23.77, 46.37 and 41.38 % respectively showing highest reduction percentage in NC group as 46.37%. The mean number of liver granulomas in the same groups was 5.40±2.7, 3.13±0.822, 2.65±1.20 and 3.76±1.39 respectively with the highest reduction percentage in NC group as 50.9% (Table 3, Graph 4).

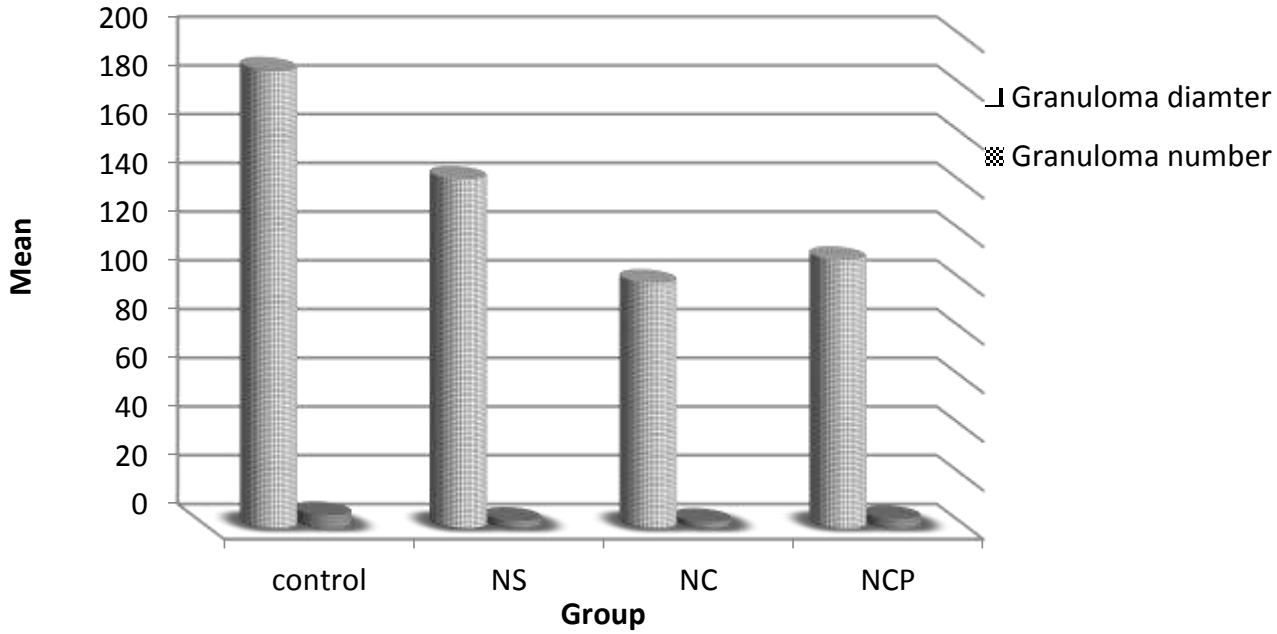
DISCUSSION

N. sativa has a remarkable anti-oxidant and anti-helminthic activity with a considerable reduction in the total number of recovered worms and the possible

Table 3. Effect of treatment on hepatic granuloma in NS, NC and NCP groups compared to control experimental mice.

Group	Granuloma diameter (Reduction %)	Granuloma number (Reduction %)
	Mean±SD	
Control	187.86±31.5	5.40±2.7
NS	143.20±35.5 (R 23.77%)	3.13±0.822 (R 42%)
NC	100.74±25.1 (R 46.37%)	2.65±1.20 (R 50.9%)
NCP	110.12±24 (R 41.38%)	3.76±1.39 (R 32%)

i) P<0.001(highly significant) in comparing mean granuloma diameter between NC, NS, NCP groups, post HOC analysis revealed that the statistically significant difference was between NS, NC, and NS, NCP groups. ii) P<0.001 (highly significant) in comparing mean granuloma diameter between control and NCP, control and NS, control and NC groups. iii) P<0.001(highly significant) in comparing mean granuloma number between NC, NS, NCP groups, post HOC analysis revealed that the statistically significant difference was between NC, NCP groups. iv) P<0.05 in comparing mean granuloma number between control and NCP, p<0.001 in comparing mean granuloma number between control and NS, control and p<0.01 between control and NC groups.



Graph 4. Hepatic granuloma number and diameter in NS, NC and NCP groups of mice against control group.

explanation of its lethal effect on worms is due to its content of alkaloid nigellicine (El Shenawy et al., 2008). The importance of chitosan as a drug carrier seems obvious when the successful administration of many drugs and macromolecules was enhanced by the availability of drug carriers to concentrate it at target site, thus decreasing therapeutically unused drug levels and their side effects (Grenha et al., 2010). Chitosan was synthesized by adding Na TPP which is nontoxic, multivalent and can form gels via ionic interactions so it has been used to prepare ChNPs (Kawashima et al., 1985; Werle et al., 2009).

In our study, we used *N. sativa* alone and NS/ChNPs in combination with praziquantel to highlight the importance of treatment of *S. mansoni* infections in experimental mice with NS/ChNPs which is a novel issue. The total worm burden was reduced by 66.3% in the NS group, by 77.5% in the NC group while all worms were killed in the NCP group. Our results were near to those of Ali et al. (2016) who revealed reduction in total worm burden in mice infected by *S. mansoni* by 57% when treated by *N. sativa* oil alone and by 47% when treated by *N. sativa* oil and *Chroococcus turgidus* algae. Regarding the female and male worm burdens, in the present work there are reductions in both by 82 and 55% respectively in NS group and by 100 and 66% respectively in NC group coinciding with the results of Ali et al. (2016), who revealed reduction of both female and male worm burdens by 41 and 64% respectively in *N. sativa* oil treatment while in contrast to combined treatment which showed reduction by 21 and 49% respectively, this highlights the importance of loading *N. sativa* on ChNPs

in our study in addition to the enormous results achieved by combination with praziquantel which caused depletion of all worms. Our results were higher than Abououf et al. (2018) who used *N. sativa* only and combined with praziquantel against mature *S. mansoni* in mice revealing reduction percentage in female, male, couple and total worm burdens as 65.9, 50, 68.6 and 57.5% respectively in their group which was treated by *N. sativa* only and reductions were 89.2, 97.5, 91.6 and 93.7% respectively for the same worm burdens in their group which was given combined treatment of praziquantel and *N. sativa* oil; proving highly effective NS/ChNPs in our study which showed 100% reduction in all worm burdens when combined with praziquantel. On comparing efficacy of black seeds (*N. sativa*) oil on other parasitic infections like *Hymenolepis nana* in infected mice, results revealed there was a significant decrease in the mean number of eggs per gram of feces by 88.85% at 7^{th} day after treatment with 5 ml/kg and by 100% at 14^{th} day of treatment. This was proof of the wide scaled efficacy of *N. sativa* in parasitic infections (Al-Megrin, 2016).

In the current study, NS/ChNPs affects greatly the oviposition of female worms with decreased immature eggs in oogram pattern from 52% in control group mice to 4% in NC group while there were increased dead eggs from 3.4% in control group mice to 32.5% in NC group indicating affection of fecundity of female worms by NS/ChNPs. These observations were also reported by Utzinger et al. (2002), Suleiman et al. (2004) and Mati et al. (2010), as they found that reduction in the worm burden and egg density in treated mice was considered as a strong indication of the effectiveness of anti-schistosomal agents. In the study of Abououf et al. (2018) by using *N. sativa* oil alone against mature worms, the dead eggs increased from 5.25 to 21.4% of oogram pattern and immature eggs decreased from 62.5 to 31% of oogram in the untreated mice group while our results relying on NS/ChNPs achieved more treating effects as mentioned above, thus proving the highly effective ChNPs as drug delivery system in treatment of *S. mansoni* mature worms. In other study, dead eggs increased from 9 to 16% of oogram in *S. mansoni* infected mice when treated with a mixture of *N. sativa* oil and aqueous garlic extract and immature eggs decreased from 54 to 20% of oogram pattern in the same group of mice (El Shenawy et al., 2008). On comparison with the study of Metwally et al. (2018) by using other treating substances like garlic and allicin, dead eggs increased from 6.1 to 7.5% for garlic and 8% for allicin in oogram pattern while immature eggs decreased from 54.5 to 47.9% for garlic and 46.4% for allicin in oogram. *In vivo* activity experiments of Epiisopiloturine against adult *S. mansoni* revealed increase in dead eggs from 2 to 36% in oogram pattern while immature eggs decreased from 79 to 24% when a dose of 40 mg/kg was given 45 days post-infection (Guimarães et al., 2015).

In the group treated with combined therapy of *N. sativa* with praziquantel, dead ova increased from 3.4 to 64.1% in oogram pattern while immature ova were completely disappeared and this coincided with the study of Abououf et al. (2018).

On using praziquantel alone in treatment of mice infected with *S. mansoni*, the dead eggs increased from 12 to 81.1%, using *N. sativa* only dead eggs increased from 12 to 17.8% and using praziquantel combined with *N. sativa,* the dead eggs increased from 12 to 95.8% in oogram pattern (Mahmoud et al., 2002). These results were higher than our study revealing that praziquantel in combinations improved the treating abilities of *N. sativa* oil while NS/ChNPs without being combined with praziquantel have the second higher treatable effects.

In our study, NS/ChNPs showed a reduction in liver egg load by 84.5% and intestine egg load by 67.8% from the control mice. This significant reduction was higher than that of Abououf et al. (2018) who recorded reduced liver egg load by 57.8% and intestine egg load by 81.4% when *N. sativa* oil was used alone indicating shift of NS/ChNPs to liver tissue. Also, these results coincided with those of Ali et al. (2016) who showed significant reduction in number of eggs per gram liver tissue when treated with algal extract, *N. sativa* oil and both by percentages of 56, 65 and 74% respectively, while the reduction percentages in intestine egg loads were 47, 44 and 62% of the same treated groups respectively. At the same time, our results achieved higher treating results than Mahmoud et al. (2002) who showed reduction in liver and intestine egg loads in mice treated by *N. sativa* alone by 33.7 and 33.2% respectively, while when combined with praziquantel of different doses, reductions were by 77.1 - 80.7% and 93.8 - 92.9% in liver and intestine egg loads respectively. Also, Mahmoud et al. (2002) results proved that away from combinations with praziquantel, our NS/ChNPs have the upper hand in treating mice infected with *S. mansoni*.

Regarding the hepatic granuloma formed by *S. mansoni*, the present study revealed high effective treating abilities of NS/ChNPs on reducing the granuloma diameter by 46.3% and granuloma number by 50.9% from the control group while when combined with praziquantel, it showed lower results by reduction percentages of 41.3 and 32% respectively from the control indicating the high efficacy of NS/ChNPs in improvement of bilharzial hepatic changes. These results were different from Mahmoud et al. (2002) who revealed that the combination of praziquantel with *N. sativa* did not improve the hepatic granuloma diameter. Abououf et al., (2018) showed a reduction in granuloma diameter by 26.7% when treated with *N. sativa* oil, thus proving the high effect of NS/ChNPs but the reduction in granuloma number had nearly the same effect (51%). Comparing our results with those of Sheir et al. (2015) who showed reduction in granuloma diameter when mice treated by combined *N. sativa* with artemether and/or praziquantel to 35.4 and 32.2% respectively, we found that effective

action was in the use of NS/ChNPs in our study.

Due to the ability of nanostructures to cross the cell and tissue barriers as a result of their very small size, they are considered now as a wide base in the biomedical sciences (Dou et al., 2016). Characters of nanostructures that make them more distinctive than the ordinary drug delivery systems include high specificity, high drug-carrying capacity, high stability, possibility to use different routes of administration and ability for controlled release (Pal et al., 2011). Chitosan has anti-bacterial and anti-fungal properties being soluble in diverse acids and able to form gels and complexes, and is also safe and non-toxic (Kim and Rajapakse, 2005). Moreover, ChNPs when used in mice infected with *S. mansoni*, produced 47% protection suggesting an important role in inducing immune response protecting against schistosomiasis (Oliveira et al., 2012).

Regarding the toxicity and safety of chitosan, there was no published data inquiring about safety of chitosan for human use or providing data about human toxicity of chitosan-based formulations. However, good safety was proved by several animal toxicity studies (Mohammed et al., 2017). Degradation of chitosan depends on the degree of its deacetylation and it is approved safe to be used in dietary consumption and for wound dressing (Wang et al., 2011).

Conclusion

This study thus revealed that NS/ChNPs is a novel anti-schistosomal combination that gives unique results in the elimination of adult schistosomal worms and hepatic granuloma size-reduction whether alone or combined with praziquantel.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Review

The insight and survey on medicinal properties and nutritive components of Shallot

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Shallot is a horticultural commodity belonging to spice vegetables. Shallot (*Allium ascalonicum* L.) is a perennial crop which is grown as an annual for its cluster of small cloves and bulbs. Persian shallot also is native and endemic of Iran and grows as a wild plant across Zagross mountains at high elevations. Shallot is an important source of carbohydrate, vitamin A, B, and C. Phenolic compound in Shallot consist of gallic acid, apigenin, eriodictyol, quercetin, isoquercetin, rutin, kaempferol, catechin and tannic acid. The most important health benefits of shallots are reduction of cancer risk, improve heart health, aid detoxification, help control diabetes, improve brain health, help to fight obesity and treat allergies, boost bone health, maintain vision health, boost immunity, improve skin health, increase abdominal health and keep hair healthy. The dominants medicinal properties of Persian shallot is it antibiotic, hypolipidemic, anticancer, antioxidant, hypoglycemic, kidney protective and hepatoprotective properties. This review article allowed verifying shallots as sources of compounds with valuable nutritional and bioactive properties with great ability for incorporation into foods with functional properties. Also, treatment with natural herbal medicine like shallot as non-synthetic drug is recommended.

Key words: Medicinal properties, nutritive components, shallot.

INTRODUCTION

Both natural products and traditional medicines have great importance (Shahrajabian et al., 2019a, b, c; Sun et al., 2019). Traditional medicine refers to health practices, knowledge, approaches and beliefs incorporating plants and herbs based on both ancient and modern pharmaceutical science (Ogbaji et al., 2018; Shahrajabian et al., 2019d,e). Traditional Asian medicine plays an important role in sustainable agriculture and food systems; it also offers a holistic and significant approach to prevent diseases while making suitable usage of organic and herbal products (Soleymani and Shahrajabian, 2012; Ogbaji et al., 2013; Ge et al., 2018; Shahrajabian et al., 2018; Soleymani and Shahrajabian, 2018).

SHALLOT OCCURRENCE AND CULTIVATION

Shallot is one of the most important vegetable crops in

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various local cuisines in different part of the world (Sulistio et al., 2015; Yao et al., 2017; Tabor, 2018). Shallot (*Allium ascalonicum* L.) is a perennial crop which is grown as an annual for its cluster of small bulbs or cloves. Shallots are valuable spices for both flavoring dishes and as medicinal plants (Swamy and Veere Gowda, 2006). Greek history and literature mention shallots. It most likely originated in Southeast Asia and from there, spread into India and the Mediterranean region. Shallot is a hardy member of the onion family that is famous for its delicate, meaty, onion-like flavor. Persian shallot, a bulb producing plant from Alliaceae, is a wildly growing plant collected for its bulbs, and it is called Mooseer in Farsi, are oval, white skinned and completely different from common shallot (*Allium ascalonicum*) (Ebrahimi et al., 2019). Persian shallot is native and endemic of Iran and grows as a wild plant across Zagross mountains at high elevations of different provinces from Northwestern to Southern of Iran with the climate of very cold to moderate cold (Moradi et al., 2013). Shallot is a major component of many Asian diets and is widely believed to be beneficial to health (Jalal et al., 2011). Tesfa et al. (2015) found that shallot can be a substitute where bulb onion does not do well, however, the production of shallot can be limited due to poor soil fertility, lack of improved production techniques, unimproved varieties and high post-harvest losses. Shallots are a unique vegetable that is used by domestic consumers as every day seasoning, raw materials of food industry, and medicine (Sukasih, 2015). The most common diseases of shallots are downy mildew, bacterial soft rot and neck rot, and the most important insects are onion maggot and onion thrips.

CHEMICAL CONSTITUENTS AND HEALTH BENEFITS

Shallot is a source of carbohydrate, vitamin A, B, and C. Fasihzadeh et al. (2016) noted that 1-Butene,1-(methylthio)-(Z) (18.21%), methyl methylthiomethyl disulfide (8.41%), dimethyl tetrasulfide (6.47%), and piperitenone oxide (4.55%) are the most abundant components of Persian shallot and comprised 37% of the essential oil. Ebrahimi et al. (2008) showed that Iranian shallot landraces are important in mineral elements and essential fatty acids content and are recommended for human nutrition. Sittisart et al. (2017) showed that shallots extracts contained some polyphenols such as apigenin, gallic acid, catechin, quercetin, kaempferol and tannic acids which are famous compounds possessing antifungal activity. Golubkina et al. (2019) indicated that shallot is an excellent candidate for the health-centered strategy of producing functional foods with high levels of Se and antioxidants; and the usage of arbuscular mycorrhizal fungi and selenium application represent environmentally friendly strategies to enhance the overall yield and quality performances of shallot bulbs.

Fattorusso et al. (2002) reported two new furostanol saponins, named ascalonicoside A1/A2 (1a/1b) and ascalonicoside B(4), respectively, along with compounds 2a and 2b. Phaiphan et al. (2019) discovered that heating and shallot supplementation can massively improve the quality of apple juice. Yin et al. (2006) suggested the use of shallot and scallion oils in food systems which may enhance lipid and microbial stability. Raeisi et al. (2016) concluded that the application of 3% ajwain seed extract gave the best antioxidant and antimicrobial activities, as well as sensory, up to 15 days of storage, followed by 3% shallot fruit extract. Leelarungrayub et al. (2006) stated that organic solvent and aqueous extracts of garlic and shallot bulbs had significant antioxidant potential, as measured by decreases in free radicals and an ability to inhibit lipid oxidation. Wongmekiat et al. (2008) indicated the protective potential of shallot extract against CsA nephrotoxicity and suggest a significant contribution of its antioxidant property to this beneficial effect. Abdelrahman et al. (2017) provided evidence for the anticancer from shallot plants and a strong foundation for more investigations to build theoretical bases for cell apoptosis and development of novel anticancer drugs. Seyfi et al. (2010) proved that shallot is a useful herb with therapeutic or preventive activity against angiogenesis related disorders. Chen et al. (2011) have shown the potential of shallots for use in treating adenoviral infection activities. Krejcova et al. (2014) found the usage of Persian shallot for the treatment of inflammatory disorders. They introduced 2-[(Methylthio)methyldithio] pyridine *N*-oxide with high anti-inflammatory effects. Hajian et al. (2018) showed that shallot extract can dose dependently reduce the factors related to lead induced renal damages. Falahati et al. (2011) indicated that crude juice of shallot has anti-candidal activity and might be promising in the treatment of candidiasis. Kongkaew and Phichai (2010) found that dried shallot powder, was effective at inhibiting the growth of *Trichoderma* spp. isolated from Yanagi mushroom. Noengpa (2004) mentioned that water extract of shallot showed inhibitory effects on C. *gloeosporioides* and *Fusarium* sp. spore growth. Amin et al. (2009) noticed that based on the antimicrobial compounds, shallot can be effective medicine for treatment of dermatomycosis and other infectious diseases. Jalal et al. (2011) found that Iranian shallot extracts appear to improve learning and memory impairments in fructose-fed rats. Mohammadi-Motlagh et al. (2011) indicated that shallot can be a candidate for prevention and treatment of many diseases related to inflammation and malignancy. Leelarungrayub et al. (2004) indicated that hexane-extract shallot had very high activity on protecting the human erythrocyte from radicals and is possible to be modified for medical plants or commercial product in the future. Sadat Hosseini et al. (2017) found that the Persian shallot extract could be considered as a potential candidate for production of drug for the prevention or treatment of human hepatoma.

Table 1. Contents of polyphenols in extracts of chili, shallot and garlic (Sisaket varieties) (Sittisart et al, 2017).

Phenolic compound	Plant extract (mg/mL)		
	Chili	Shallot	Garlic
Gallic acid	32.77	2.13	3.14
Eriodictyol	-	0.37	-
Apigenin	11.49	0.11	0.32
Isoquercetin	2.82	10.55	0.33
Kaempferol	-	0.66	-
Quercetin	-	35.91	-
Rutin	3.22	-	-
Catechin	8.50	-	6.93
Tannic acid	66.33	21.71	13.18

Table 2. Some components found in Persian shallot (Moradi et al., 2013).

S/N	Component
1	Allicin
2	Saponins
3	Sapogenins
4	Ajoene
5	Sulphuric compounds (thiosulfinates)
6	Flavonoids: Quercetin and Kaempferol
7	Mineral Elements
8	Essential fatty acids
9	Folic acid
10	Protein
11	Fiber
13	Vitamin C

Iranian shallot extracts appear to improve learning and memory impairments in fructose-fed rats (Razieh et al., 2011). Amanzadeh et al. (2006) proved the inhibitory effect of Persian shallot hydroalcoholic extract on *Leishmania infantum*. Nasiri Kashani et al. (2009) indicated that shallot crude juice has antifungal activity and looks promising to be an alternative for chemical antifungal agents that have sometimes serious effects. Rattanachaikunsopon and Phumkhachorn (2009) reported that shallot oil inhibit pathogenic bacteria including *Bacillus cereus*, *Camplobacter jejuni*, *Escherichia coil O 157:H7*, *Listeria monocytgenes*, *Salmonella enterica*, *Staphylococcus aureus*, and *Vibrio Cholerae*. Farajii et al. (2018) stated that the shallot extract was preferred in both terms of reducing microbial growth and suitable sensory properties. Zarei Mahmoudabadi and Gharib Nasery (2009) concluded that the fresh crude juice of shallot bulbs has markedly anti-fungal effect, and also shallot extract has more anti-saprophytes effect at 0.25% followed by *C. albicans* and dermatophytes.

Kazemian et al. (2017) noted that hydroalcoholic shallot extract increases the number of germ cells in mice tested and helps amplify the sexual ability of male mice. Shallot as traditional medicine are for febrifuge, diabetes, blood sugar and blood cholesterol, and also prevents thickening and hardening of the blood vessels and ulcers (Sukasih, 2015). Sukasih (2015) also reported that shallot powder is widely used as an industrial raw material such as in snacks production, seasoning in cooking, and medicine. Persian shallot has been reported to have a range of health benefits which include anticarcinogenic, hypoglycemic, hypolipidemic, antioxidant, antibiotic properties, and kidney and liver protective effects (Moradi et al., 2013). Contents of polyphenols in extract of chili, shallot and garlic are shown in Table 1. Some components found in Persian shallot are presented in Table 2. Medicinal properties of in Table 3. Volatile organic compounds in shallot with absorption on SPME fiber at 20°C are presented in Table 4.

Table 3. Medicinal properties of Persian shallot (Moradi et al., 2013).

S/N	Properties
1	Antibiotic properties
2	Hypolipidemic properties
3	Anticancer properties
4	Antioxidant properties
5	Hypoglycemic properties
6	Kidney protective properties
7	Hepatoprotective properties

Table 4. Volatile organic compounds in shallot with absorption on SPME fiber at 20°C (D'Auria and Racioppi, 2017).

Compound	r.t. (min)	KI	Area (%)
Methanethiol	1.61	500	0.46±0.01
Propanethiol	2.30	600	4.20±0.02
Thiopropanal *S*-oxide	4.37	740	
2-Methyl-2-pentenal	5.66	804	0.13±0.01
2,5-Dimethylthiophene	7.18	865	1.06±0.01
Methylisopropyldisul-phide	7.78	880	2.74±0.02
Dipropyldisulphide	11.64	1094	58.57±0.05
Allylpropyldisulphide	11.82	1098	13.27±0.05
Methyl propylthiosul-fonate	12.35	1154	0.46±0.02
Dipropyltrisulphide	15.52	1294	6.99±0.03
Allypropyltrisulfide	15.74	1309	0.83±0.01

Table 5. Volatile organic compounds in shallot with absorption on SPME fiber at 50°C (D'Auria and Racioppi, 2017).

Compound	r.t. (min)	Area (%)
Propanethiol	2.31	2.57±0.01
2-Methyl-2-pentenal	5.65	0.20±0.01
2,5-Dimethylthiophene	7.18	0.51±0.01
Methylpropyldisulphide	7.77	1.42±0.03
Dipropyldisulphide	11.62	34.80±0.05
Allypropyldisulphide	11.79	7.14±0.03
Methylpropylthiosulfonate	12.36	1.72±0.02
Dipropyltrisulphide	15.66	21.70±0.05
Allypropyltrisulphide	15.82	9.18±0.03
Compound 4	19.41	4.35±0.03
Compound 5	19.64	2.00±0.02
Compound 6	19.80	3.72±0.02
Compound 7	22.51	2.02±0.02

Volatile organic compounds in shallot with absorption on SPME fiber at 50°C are presented in Table 5. The most important health benefits of shallots are shown in Table 6.

Conclusion

Shallot is a key part of diet of many populations and there is long-held belief in their health enhancing properties.

Table 6. The most important health benefits of shallots.

S/N	Benefit
1	Reduction of cancer risk
2	Improve heart health
3	Aid detoxification
4	Help control diabetes
5	Improve brain health
6	Help fight obesity
7	Help treat allergies
8	Boost bone health
9	Might maintain vision health
10	Boost immunity
11	Improve skin health
13	Enhance abdominal health
14	Keep Hair healthy

Historically, the shallot has been used for both its nutritional and aromatic properties in Iranian, Indian, Chinese, Asian, French and Mediterranean cooking. The shallot is considered an important plant in Asian medicinal practices and is commonly prescribed as an effective remedy for various ailments in Ayurvedic medicine. Shallots, like onions, are a member of the allium family, but their flavor is richer, sweeter, yet more potent. The most important benefits of shallots are high source of antioxidants, improve heart health, cancer prevention, diabetes, anti-inflammatory, antimicrobial, might help fight obesity, and help to prevent or treat allergies. The demand for shallot products is increasing every year with increase population growth and food industries. More clinical studies may be required to uncover the numerous substances and their effects in shallot that contribute to public health.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Menopause disorders and their treatment in traditional medicine in Burkina Faso

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A survey was conducted in the central region of Burkina Faso to find out about women's menopause-related disorders and their treatment in traditional medicine. Fifty-six (56) species have been identified to treat different symptoms such as hot flashes, insomnia, nausea, joint and muscle pain, itching, lower abdominal pain, edema, mood disorders, vertigo. Leaves and stem bark were the most recommended in the preparation of recipes, at 43 and 39% respectively. The majority of the species (60.72%) was local food plants with a high use value (UVs ≥ 0.50). Many chemical groups including alkaloids, polyphenols, flavonoids, saponins, tannins, sterols, triterpenes, anthraquinones, carotenoids, anthracenosides, phenolic acids, coumarins, capable of reducing or eliminating these different symptoms exist in these plants. Also, the presence of several mineral elements such as Calcium (Ca), Magnesium (Mg), Phosphorus (P), Sodium (Na), Aluminum (Al), Iron (Fe), Potassium (K), Iodine (I), Vitamins A, B, C, F, K, P, E, proteins, lipids, carbohydrates, fibers, resins and gum show the importance of these plants in human nutrition. These local plants are therefore potential sources for the development of new natural nutraceuticals in the management of menopausal period in women.

Key words: Menopause, medicinal plants, nutrients, phytoestrogens.

INTRODUCTION

Menopause or stopping menstruation, refers to the period that occurs when the ovaries stop producing reproductive

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hormones. It is a natural phenomenon that usually starts around age of 50 in women (Lopes and Tremollieres, 2004). It is preceded by a period of pre-menopause which causes disturbances related to imbalances in the reproduction of natural hormones. Indeed, at first, the woman's body faces a fall of progesterone and hyper impregnation in estrogen, which can create a kind of permanent premenstrual syndrome with water retention (edema), weight gain, tense breasts, headaches, and mood disorders (irritability, aggressiveness), then in a second time, a few months or a few years later, it is estrogen deficiency that prevails with hot flashes, tiredness, genitourinary disorders, joint pain, vaginal dryness before the definitive installation of menopause (Sidibe, 2005; Löwy and Gaudillière, 2006). Menopause affects an increasing number of women around the world and is a public health problem. A follow-up of woman is necessary during this period in order to avoid complications and the occurrence of other diseases such as cardiovascular diseases, diabetes, joint and metabolic diseases (Tillier, 2005). Modern medical care is based on hormonal menopause treatments (THM) that eliminate certain symptoms (Ribot and Tremollieres, 2007). However, the use of these hormones to replace those naturally secreted by the ovaries often causes potentially serious side effects such as breast cancer, osteoporosis, heart disease including heart attacks and strokes, which are currently the leading cause of death in the world (Fournier et al., 2003; Azoulay, 2004). While the effects of menopause on women's bodies are well known in developed countries, the situation is opposite in Africa, especially in sub-Saharan Africa where management of the menopause period is almost non-existent (Cisse et al., 2008). Thus, very few African women can benefit from hormonal menopause treatments due to the unavailability and cost of these modern products. This would partly explain women's low attendance in health centers for menopause-related issues (Lombrail, 2000). Also, cultural attachment to the effectiveness of plant-based recipes means that at least 80% of rural populations living in developing countries depend on traditional medicine for their health care needs (OMS, 2013). Previous scientific works have shown that isoflavones present in some plants and recognized as phytoestrogens, are able to reduce the frequency of hot flashes and bone resorption (Lecerf, 2007). Also, some estrogens in plants are nervous sedatives against anxiety, insomnia and menopausal disorders (Nogaret, 2011). In addition, as a result of WHO's policy of promoting traditional medicine, plant medicines now occupy a considerable place in the international pharmaceutical trade (OMS, 2013). The current trend is thus converging towards health food. Indeed, many studies have proved the efficiency of nutraceuticals which are defined as foods whose specific properties go beyond the simple nutritive effect associated with the nutrients they contain (Bouyahya, 2016; Tchatchambe et al., 2017). For example, studies have shown that women with traditional soy-based diet, suffer less from the effects of menopause (Vergne and Sauvant, 2006). There are several other medicinal and nutritional plants containing many active ingredients and which have complementary or synergistic therapeutic activities capable of relieving the menopausal disorders. However, this category of plants is very little known. It is therefore urgent to explore local plants in order to develop improved traditional medicines, nutraceuticals or isolate new bioactive molecules with fewer side effects for the management of menopausal period in women.

The objective of this study was to provide scientific documentation on the plants used by women in Burkina Faso to treat menopausal disorders.

MATERIALS AND METHODS

Data collection on the treatment of menopausal disorders in traditional medicine

The survey was conducted from February to March 2018 among women in households, at the level of their women's groups or associations in Ouagadougou, Pabre and surrounding villages as well as among some traditional healers identified through networks of traditional healers and on the basis of information provided by local population. Midwives working in maternal and child health centers were also interviewed. The interviews were based on a pre-tested questionnaire (Martin, 1995) which included specific questions about the informant, his age, his level of knowledge about menopause, the treatment of symptoms in modern and/or traditional medicine, the local name of the plant used, the parts used, the right period of collecting the used parts, the mode of use, the approximate duration of treatment before the treated symptom disappears. The interviews were recorded using a dictaphone. A total of 161 people including 54 traditional healers (9 men and 45 women) were interviewed. The age of women was between 41 and 65 years old. Investigators equipped with GPS (GPS map 62 Garmin) with an accuracy of less than 2 m visited and geo-referenced the practice sites of some traditional healers. Photos of plants of interest were taken in Pabre and surrounding villages. Samples of these plants were collected and identified by the Botany team of Ouaga 1 Professor Joseph Ki-Zerbo University. The APG III (2009) classification system was used (Group, 2009) and a herbarium has been made.

Chemical composition of the listed plants

A bibliographic research made it possible to know chemical groups and phytonutrients present in the identified plants.

Data analysis

The importance of each species was determined by calculation of its use value *(UVs)* according to the simplified formula of Cotton and Wilkie (1996).

$$UVs = \frac{U}{N}$$

U indicate the number of uses where the plant is mentioned and N is the number of informants who mentioned the plant. Data were

treated and analyzed with SPSS software version 15. The average utilization values of the main parts of plant were compared using one-way analysis of variance (One Way ANOVA) and the differences are considered statistically significant for a value of "p less than 0.05".

RESULTS

Fifty-six (56) local species used in the treatment of various menopausal symptoms have been identified. The information received concerns the local name of the plant, the disorders treated, the used parts in traditional recipes, the period of availability of the used parts, the method of preparation and the route of administration of recipes in traditional medicine, the edible parts and the mode of preparation of food parts. The mineral elements, vitamins and chemical groups found in each plant by literature are also shown in Table 1.

Results showed that the most common symptoms experienced by women were hot flashes, insomnia, nausea, joint and muscle pain, irregular menstruation, itching, lower abdominal pain, edema, mood disorders, vertigo. Leaves and stem bark were the most commonly used in recipes preparation, at 43% and 39% respectively. Other parts such as roots, fruits, seeds and flowers were rarely used for care. Decoction as method of preparing recipes and oral route as mode of administration were the most recommended. Maceration was indicated when the stem bark is the used part. The majority of the plants (60.72%) used in the treatment of menopause disorders was also local food plants well known including *Acacia macrostachya, Adansonia digitata, Balanites aegyptiaca, Bombax costatum, Cassia tora, Cleome gynandra, Corchorus olitorius, Diospyros mespiliformis, Ficus sycomorus, Glycine max*, *Hibiscus sabdariffa , Manguifera indica, Moringa oleifera, Ocimum gratissimum*, *Parkia biglobosa, Petroselinum crispum, Psidium guajava, Sclerocarya birrea, Tamarindus indica, Vitellaria paradoxa, Zingiber officinale*, *Ziziphus mauritiana* (Table 1). They had a high use value (UVs ≥ 0.50). Fruits and leaves were the most consumed parts (41%) by populations. The leaves used in food are usually boiled and filtered and eaten or mixed with flour in the form of couscous. Certain leaves can be eaten as salad. For some recipes, they are dissolved in water and the filtrate is recovered as vinegar for the preparation of certain dishes. The fruits are consumed in their natural state for the most part but can be boiled at certain times. They can also be used as vinegar. The consumption of other parts such as calyx, seeds and rhizomes was specifically recommended for certain plants. Bibliographic data showed the presence of many chemical groups and phytonutrients in these plants including alkaloids, polyphenols, flavonoids, saponins, tannins, steroids, triterpenoids, anthraquinones, carotenoids, anthracenosides, phenolic acids, coumarins as well as numerous mineral elements such as calcium (Ca), magnesium (Mg), phosphorus (P), sodium (Na), aluminum (Al), iron (Fe), potassium (K), vanadium (V), copper (Cu), lead (Pb), manganese (Mn), selenium (Se), chromium (Cr), iodine (I). Vitamins A, B1, B2, B3, C, F, K1, P, E and proteins, lipids, carbohydrates, gum, resin, fibers were also present in these plants.

Figure 1 (1a to 1e.) show some plants used to treat the most common menopausal symptoms encountered by women.

DISCUSSION

Most of the population of Burkina Faso, a landlocked country in West Africa with an area of 274 000 km^2, lives in rural communities and relies heavily on local plant products for their daily lives. Fifty-six (56) local plants were identified in the treatment of menopausal disorders with a predominance of woody species (84%) on herbaceous forms (16%). Previous work had also reported the therapeutic importance of woody plants over herbaceous forms (Betti, 2002; Zerbo et al., 2012). The results showed that leaves and stem bark were the most recommended parts at 43% and 39% respectively in traditional recipes to treat several symptoms. Using leaves is to be encouraged because it has a double advantage, firstly because being the site of synthesis of secondary metabolites, leaves contain many chemical groups, but also because the use of leaves prevents the destruction of the plant and preserves its durability (Lumbu et al., 2005; Bi et al., 2008). However, the leaves and fruits of some plants are only available during the rainy season, which explains why traditional healers dry them so they can be used all year long. The disadvantage of this method is that, exposure of leaves and fruits to sun or their decoction causes the loss of certain active ingredients they contain. It is therefore necessary to sensitize traditional healers as well as women to practice the right harvesting methods of drying and preserving some parts of plants or in cooking the traditional vegetables in order to preserve as many phytonutrients as possible.

Previous studies have shown that gynecological and obstetric disorders are among the first three health problems in Burkina Faso (Besancenot et al., 2004; Ramde-Tiendrebeogo et al., 2019). The plants in this study would contribute to the management of certain pathologies in women. Indeed, phytoestrogens and phytosterols present in some plants are recognized for their effectiveness in reducing the frequency of hot flashes which are the most common symptom encountered in women (Vergne and Sauvant, 2006). Plants such as *A. macrostachya, A. digitata, Afzelia Africana, Annona senegalensis, B. aegyptiaca, Boswellia papyrifera*, *C. gynandra, Combretum glutinosum, F. sycomorus, Gueira senegalensis*, *M. oleifera, O. gratissimum, P. crispum* indicated in the treatment of hot flashes (Table 1) constitute a source for new phytoestrogens research. Previous studies have shown

Table 1. Plants used in the treatment of menopausal disorders.

Species and familly	Local names	Disorders treated	Used parts in medicinal practice	Edible parts in human nutrition	Average of Use value *(UVs)*	Bibliographic data	
						Chemical groups phytonutrients and other elements found in the plant	Reference
Acacia macrostachya Rchb. ex DC. Fabaceae-Mimosoideae	Zamanega	Lower abdominal pain, hot flashes, two much sweat	Leaf , Stem bark	Fruit, Leaf	0.65	Catechins, Saponins, Tannins, Alkaloids, Fe, Mg ,V, Na, Ca, Vita P	Hilou et al. (2014) and Msika et al. (2014)
Acacia nilotica L. (Wild) ex Del. Fabaceae-Mimosoideae	Peguen-daaga	Edema, general tiredness	Leaf, Stem bark , Seed	Seed	0.55	Tannins, Flavonoids, Phenolic compounds, Mg, Fe, Na, Ca	Okuda et al. (1991) and Nagumanthri et al. (2012)
Acacia gourmaensis A. Rich. Fabaceae-Mimosoideae	Gonsabelga	Articular and muscular pain, palpitations, itching	Leaf, root	-	0.55	Alkaloids, Flavonoids, Tannins	Guinko (1997) and Pawinde et al. (2008)
Acacia Senegal (L.) Willd Fabaceae-Mimosoideae	Gonpeelega	Vaginal infection, stomach aches	Leaf, Stem bark	-	0.65	Tannins, Alkaloids, Flavonoids, Saponins, Phenolic compounds	Sereme et al. (2011) and Pal et al. (2012)
Acacia seyal Delile Fabaceae-Mimosoideae	Gon-ponsego	Lower abdominal pain, edema	Leaf, Stem bark	-	0.65	Tannins, Alkaloids, Flavonoids, Saponins, Phenolic compounds	Seigler (2003) and Sereme et al. (2011)
Acacia tortilis (Forssk.) Hayne subsp. raddiana (Savi) Fabaceae-Mimosoideae		Headaches, edema	Leaf, Stem bark	-	0.60	Tannins, Alkaloids, Flavonoids, Saponins, Phenolic compounds	Seigler (2003) and Jaouadi et al. (2015)
Adansonia digitata L. Malvaceae	Tohega	Lower abdominal pain, itching, hot flashes	Fruit	Leaf, Fruit	0.65	Pectins, Coumarins, Catechins, Tannins, Ca, Fe, P, Na, Vita A, Vita B1, Vita B2, Vita B3, Vita C, Vita P, Lipids, Proteins, Carbohydrates, Citric acid, Malic acid, Oxalic acid, Mucilage	Osman (2004) and Makalao et al. (2015)
Afzelia africana Smith Fabaceae Caesalpinioideae	Kankalga	Vomiting, general tiredness, hot flashes	Stem bark	Fruit, Leaf	0.55	Mucilage, Tannins, Coumarins, Vita P, Flavonoids	Akinpelu et al. (2008) and Ejikeme et al. (2010)
Annona senegalensis Pers. Annonaceae	Barkudga	Hot flashes, two much sweat, inflammation	Leaf	Fruit	0.55	Coumarins, Tannins, Vita C, Vita P, Mucilage, Pectins	Kini et al. (2008) and Potchoo et al. (2008)
Anogeisus leocarpus (DC) Guill and Perr (Stem) Combretaceae	Siiga	Lower abdominal pain	Stem bark, Leaf	-	0.50	Glycosides, Phenols, Tannins, Saponins, Alkaloids, Steroids, Ellagic acids, Anthraquinones	Mann et al. (2008) and Shuaibu et al. (2008)
Antada africana Guill.&Perr Fabaceae-Mimosoideae	Sinnogo	Lower abdominal pain, two much sweat	Root, leaf	-	0.55	Coumarins, Flavonoids, Gallic tannins, Anthocyanidins, Sterols, Triterpenes, Carotenoids, Saponosides, Rotenone, Paucine	Diallo et al. (2001) and Cioffi et al. (2006)
Azadirachta indica (A. Juss.) Meliaceae	Neem	Knee pain, general tiredness	Leaf, Stem bark	-	0.65	Azadirachtins, Nimocinol, Isomeldenin, Azadirachtol, Isoazadironol	Sultana et al. (2007) and Atawodi and Atawodi (2009)

Table 1. Contd.

Balanites aegyptiaca (L.) Delile Zygophyllaceae	Kyegelga	Headaches, hot flashes, Two much sweat, edema	Fruit, Stem bark	Fruit, Leaf	0.65	Anthocyanins, Sterols, Triterpenes, Tannins, Saponins, Vita B1, Vita B3, Vita C, Vita E	Kini et al. (2008) and Makalao et al. (2015)
Bauhinia refuscens Lam Cesalpiniaceae	Tipoega	Hot flashes, nausea	Root, leaf, fruit	-	0.55	Anthraquinones, Resins, Flavonoids, Tannins, Saponins, Cardenolides	Usman et al. (2009) and Garbi et al. (2015)
Bombax costatum Pellegr.& Vuillet Malvaceae	Voaka	Insomnia, lower abdominal pain, two much sweat	Flower	Calyx, Leaf	0.70	Para-coumaric acid, Anthocyanins, Vita P, Vita E, Mucilage	Guinko and Pasgo (1992) and Nenonene et al. (2009)
Boswellia papyrifera Hochst Bruseraceae	Kombre-yongo	Vaginal infection, hot flashes	Leaf, Stem bark	-	0.55	Phenolic compounds, Alkaloids, Saponins	Abdallah et al. (2009) and Paul et al. (2012)
Cassia tora Linn. Fabaceae-Caesalpinioideae	Sogoda	Itching, lower abdominal pain, two much sweat	Leaf	Leaf, Fruit	0.65	Xanthones, Flavonoids, Vita A, Vita B1,Vita B2, Vita B3, Vita C, Mucilage	Kim et al. (2004) and Phongpaichit et al. (2004)
Capparis sepiaria Linn. Capparidaceae	Kalyanga	Stomach aches, palpitations, insomnia, nausea	Stem bark	Fruit, Leaf	0.50	Flavonoids. Alkaloids. Steroids, Tannins, Anthraquinones, Resin, Carbohydrates, Gum, Ca, Mg, Vita C, Proteins	Mishra et al. (2007) and Rajesh et al. (2010)
Cleome gynandra Linn. Cleomaceae	Kiennebdo	Hot flashes, general tiredness, itching	Leaf	Leaf	0.65	Para-coumaric acid, Gallic acid, Vanillin, Caffeic acid, Fe, Vita F	Muchuweti et al. (2007) and Anbazhagi et al. (2009)
Combretum micranthum G.Dom. Combretaceae	Randga	Osteoarthritis, itching	Leaf	-	0.65	Saponins, Tannins, Glycosides flavonoids, Alkaloids, Glycosylflavones, Flavans, Resins	Chika and Bello (2010) and Udoh et al. (2012)
Combretum glutinosum Perr.Ex DC Combretaceae	Kouenga	Hot flashes, eczema	Leaf, Stem bark	-	0.55	Sterols, Triterpenes, Flavonoids, Taninns, Saponins, Coumarins	Harouna et al. (2012) and Yahaya et al. (2012)
Corchorus olitorius L. Malvaceae	Bulvaka	Stomach aches	Leaf	Leaf	0.65	Quercetin, Caffeoylquinic acid, Glycosids, Vita A, Vita B2, Vita B3, Vita C, Mucilage	Azuma et al. (1999) and Ndlovu and Afolayan (2008)
Crataeva adansonii DC. Capparidaceae	Kalguem-tohega	Vaginal infection, stomach aches, itching, vomiting	Leaf	Leaf	0.60	Tannins, Flavonoids, Triterpenoids, Vita A, Vita B2, Vita C	Ahama et al. (2010) and Agbodan et al. (2017)
Daniella olivieri (Rolfe) Fabaceae - Caesalpinioideae	Aoga	Two much sweat, insomnia	Leaf, stem bark	-	0.55	Alkaloids, Steroids, Phenol, Tannins, Phylate, Oxalate Saponins, Na, K, Ca Mg, Zn, Fe Pb	Onoja et al. (2015) and Temitope et al. (2016)
Diospyros mespiliformis Hochst. ex A.DC. Ebenaceae	Gaaka	Vomiting, stomach aches, headaches	Stem bark, Fruit	Fruit, Leaf	0.65	Pectins, Tannins, Lupeol, Lupenone, Betulin, Betulinic acid , Vita P	Kini et al. (2008) and Mohamed et al. (2009)
Faiderbia albida (Del) a. Chev Fabaceae-Mimosoidae	Zaanga	Arthrose, stomach aches	Leaf, Stem bark	-	0.60	Alkaloids , Saponins, Tannins	Tijani et al. (2008) and Salawu et al. (2010)
Feretia apodanthera Del. Rubiaceae	Kitinga	Infections, edema, insomnia	Root bark, Leaf	-	0.50	Iridoids. Tannins Saponins, Steroids Triterpenes	Taiwe et al. (2016) and Owolabi et al. (2018)

Table 1. Contd.

Ficus thonningii Blume Moraceae	Kuusga	Vomiting, general tiredness	Leaf, Stem bark	-	0.55	Anthraquinones, Flavonoids, Alkaloids, Saponins, Tannins, Carbohydrates	Otimenyin et al. (2004) and Usman et al. (2009)
Ficus sycomorus L. (Moraceae)	Kankanga	Hot flashes, osteoarthritis, nausea	Leaf, Stem bark	Fruit	0.65	Alkaloids, Phenols, Carbohydrates, Flavonoids, Saponins, Steroids, Tannins, Triterpenoids, Anthracenosides, Anthocyanins, Coumarins, Acide 3-hydroxybenzoic, Acide 4-Hydroxybenzoic, Fe, Ca, Vita C, Vita A, Vita B_1, Vita B2, Vita B3, Proteins, Fibers	Kerharo and J-G (1974), Nongonierma et al. (2005), Abdel-Hameed (2009) and Ramde-Tiendrebeogo et al. (2012)
Gardenia erubescence Stapf & Hutch. Rubiaceae	Subudga	Vaginal infection, nausea	Root	Fruit	0.45	Phenolic compounds, Tannins, Flavonoids, Ca, Mg, K, Na Mn, Fe , Zn, C	Bello et al. (2008), Lamien-Meda et al. (2008) and Ouédraogo et al. (2019)
Gardenia soketensis Hutch Rubiaceae	Tang-rakweenga	Headaches, nausea, itching	Roots, leaf		0.50	Alkaloids, Steroids, Glycosides, Saponins, Flavonoids, Tannins	Jansen et al. (2008) and Jodi et al. (2008)
Glycine max (L.) Merr. (soja) Fabaceae	Soja	Hot flashes, rheumatism	Leaf, Stem bark	Seed	0.70	Proteins, Flavonoids, Carotins, Anthocyanins, Isoflavones glycosides, Phytoestrogens (Genistein, Daidzein), Fe, Mg, Ca, K, Mn, Na, Cu, Zn Se, Vita A, Vita B1, Vita B2, Vita B6, Vita C, Vita D, Vita E	Kudou et al. (1991), Plaza et al. (2003), and Barhe and Tchouya (2016)
Gueira senegalensis J.F. Gmel Combretaceae	Wiliwinga	Hot flashes, vaginal infection	Stem bark	-	0.65	Phenolic compounds, Flavonoids, Tanins	Zhigila et al. (2015) and Sulaiman (2016)
Hibiscus sabdariffa L. Malvaceae	Biito, Wegdo	Weight gain, vertigo, nausea	Leaf	Calyx, Leaf	0.70	Malic acid, Oxalic acid, Mucilage, Ca, Fe, P, Vita B1. Vita B3, Vita P, Vita C, Citric acid, Pectins	Ali et al. (2005) and Cisse et al. (2009)
Lannea acida A. Rich Anacardiaceae	Sabtulga	Vaginal infection, edema	Stem bark	-	0.55	Phenolic compounds, 6,7-(2",2"-dimethyl chromeno)-8-γ,γ-dimethyl allyl flavanone, 3',4'dihydroxy-7,8 (2",2"-dimethyl chromeno)-6-γ,γ - dimethyl allyl flavonol, 7-methyltectorigenin Irisolidone, Tannins	Ouattara et al. (2011) and Muhaisen (2013)
Lannea microcarpa Engl. et K. Krause Anacardiaceae	Sabga, Siibi	Stomach aches, menstrual irregularities, insomnia	Leaf, Stem bark	Fruit, Leaf	0.60	Xanthons, Tannins, Terpenoids. Steroids, Anthocyanins, Flavonoids, Vita C, Vita A	Kini et al. (2008) and Ajiboye et al. (2013)
Leptadenia hastata (Pers.) Decne. Asclepiadaceae	Lelongo	Articular and muscular pain	Leaf	Leaf	0.65	Tannins. Favonoids. Proanthocyanidins, Saponins, Alkaloids, Al, Ca, Fe, V, Vita A, Vita C	Freiberger et al. (1998) and Bello et al. (2011)
Maerua angolensis DC (Forsk) Capparidaceae	Zilgo	Stomach aches	Stem bark	Leaf	0.45	Tannins, Steroids, Cardiac glycosides, Flavonoids, Terpenoids, Ca, Vita C	Ondiek et al. (2010) and Ayo et al. (2013)

Table 1. Contd.

Manguifera indica L. Anacardiaceae	Manguier	Weight gain	Stem bark, Leaf	Fruit	0.70	Flavonoids, Kinic acid Triterpenoids, Mangiferin, Xanthones, Isomangiferin, Tannins, Protocatechic acid, Catechin, Shikimic acid, Triterpenoids, Gallic acid, β-carotene, Vita C, Dehydroascorbic acid, Alanine, Glycine, γ-Aminobutyric acid	Anila and Vijayalakshmi (2002), Ribeiro et al. (2007) and Shah et al. (2010)
Moringa oleifera L. Moringaceae	Arzan-tiiga	Hot flashes, vertigo, insomnia	Leaf, Fruit, Stem bark	Leaf	0.65	Para-coumaric acid. Zeatin. Quercetin. β – sitosterol. Tannins, Caffeoylquinic acid, Kaempferol, Ca, Cr, Fe, P, V, Vita A, Vita B1, Vita B2, Vita B3,Vita C	Movo et al. (2011) and Rani and Arumugam (2017)
Ocimum gratissimum L Lamiaceae	Basilic	Hot flashes, Itching, vertigo	Leaf	Leaf	0.60	Thymol, Tert-Butanol, O-cymene, Flavonoids, Carbohydrates, Tannins, Ca, P, Na, K, Fe, Vita A, Vita B, Vita C, Vita K	Peter (2004) and Green et al. (2012)
Parkia biglobosa (Jacq.) R. Br. ex G. Don Fabaceae-Mimosoideae	Roaaga	Stomach aches, vertigo, allergy	Leaf, Stem bark	Fruit	0.65	Cardiac glycosids. Tannins, Alkaloids, Saponins, Steroids, Ca, Fe, Vita A, Vita B1, Vita B2, Vita C	Ajaiyeoba (2002) and Makalao et al. (2015)
Petroselinum crispum (Mill.) Fuss Apiaceae	Persil	Articular and muscular pain, rheumatism, hot flashes	Leaf	Leaf	0.65	Flavonoids. Tannins. Carbohydrates, Steroids, Saponins, Terpenoids, Ca, P, Na, K, Fe, I, Mn, Vita A, Vita B, Vita C	Green et al. (2012) and Wuyts (2012)
Piliostigma reticulatum (DC.) Hochst. Fabaceae-Caesalpinioideae	Baguende	Stomach aches, insomnia	Leaf, Stem bark	Leaf	0.65	Saponins, Tannins, Phlobatinins, Glycosids, K, Vita C, Vita P	Awe and Omojasola (2009) and N'Guessan et al. (2015)
Psidium guajava L. Myrtaceae	Goyaka	Edema, menstrual irregularities, lower abdominal pain	Leaf, fruit	Fruit	0.65	Glutamic acid, Asparagine, Malonic acid, Trans-aconitic acid, Cis-aconitic acid, Gallic acid, Tannins, Catechin, Xanthine, Quercetin, Lectins, Epicatechin, Uvaol, Carotenoids, Saponins, Triterpenes, Flavonoids, Ellagic acid, Guaianoic acid, Leucocyanidin, Amritoside, β –sitosterol, Vita A, Vita C ,Citric acid, Acetic acid	Wu et al. (2009) and Barbalho et al. (2012)
Saba senegalensis (A. DC) Pichon Apocynaceae	Weeda	Itching, lower abdominal pain, weight gain	Leaf	Fruit	0.65	Citric acid, Steroids, Malic acid, Terpenoids, Carotenoids, Ca, Vita A, Vita C	Kini et al. (2008) Boamponsem et al. (2013)
Sarcocephalus latifoluis (Sm.) E.A.Bruce Rubiaceae	Guinga	Anxiety disorders	Leaf, root	-	0.55	Alkaloids, Tannins, 21-O methyl-strictosamide aglycone, 21-O-ethylstrictosamide aglycone, Carbohydrates, Cardiac glycosides, Anthraquinones, Steroids, Saponins, Flavonoids	Abreu and Pereira (2001) and Arome et al. (2014)

Table 1. Contd.

Securidaca longepedunculata (Fresen.) Polygalaceae	Pelga	Edema, insomnia	Stem bark	-	0.45	Methyl 2-hydroxybenzoate (methyl salicylate), Methyl 4-hydroxybenzoate, Methyl 2-hydroxy-6-methoxybenzoate Xanthone	Jayasekara et al. (2002) and Dibwe et al. (2012)
Sclerocarya birrea (A.Rich.) Hochst. Anacardiaceae	Noabga	Stomach aches, mood disorders	Leaf, Stem bark	Fruit	0.65	Citric acid, Malic acid, Catechins, Tannins, Ca, Mg, K, P Vita C	Glew et al. (2004) and Mariod and Abdelwahab (2012)
Tamarindus indica L. Fabaceae-Caesalpinioideae	Pusga	Stomach aches, vaginal infection, nausea	Stem bark	Fruit, Leaf	0.65	Pectins, Tannins, Triterpenes, Flavonoids, Anthocyanins, K, Vita C, Vita K3, Vita P, Citric acid, Malic acid	Kuru (2014) and Makalao et al. (2015)
Vernonia amygdalina Delile Asteraceae	Koa safani	Weight gain	Stem bark, Leaf	-	0.60	Sesquiterpene lactone. Flavonoids, Terpenoids. Saponins, Tannins, Flavonoids, Reducing sugar	Akinpelu (1999) and Ayoola et al. (2008)
Vitellaria paradoxa C.F.Gaertn. Sapotaceae	Taanga	Stomach aches, weight gain	Stem bark	Fruit	0.65	Para-coumaric acid, Gallic acid, Catechins, Epigallocatechins, Cinnamic acid, Vita E, Vita F	(Maranz et al., 2003; Makalao et al., 2015)
Vitex doniana L. Lamiaceae	Aadega	Insomnia, mood disorders	Stem bark	Leaf, Fruit	0.55	Saponins, Tannins, Anthraquinones, Flavonoids, Terpenoids, Alkaloids, K, Na, Ca, Fe P, Mg, Cu, Vita A, Vita B1, Vita B2, Vita B6, Vita C	Agbafor and Nwachukwu (2011) and Vunchi et al. (2011)
Ximenia americana L. Olocaceae	Leenga	Amenorrhea, mood disorder	Stem bark, Leaf	Fruit	0.55	Cyanogen, Gallic acid, Sambunigrine, β-glucogalline, Quercetin and derivatives, Na, K, Mg Ca, Fe, P, K, Cu, Mn, Vita C , Lipids, Proteins, Sugars	Le et al. (2012), Sarmento et al. (2015) and Almeida et al. (2016)
Zingiber officinale Roscoe Zingiberacae	Gnamaku	Rheumatism, stomach aches, mood disorder	Rhizomes	Rhizomes	0.60	Polyphenols, Tannins, Flavonoids, Proteins, Mn, Fe, Ca, P, Cu	Ali et al. (2008) and Prakash (2010)
Ziziphus mauritiana Lam. Rhamnaceae	Mugunuga	Stomach aches, weight gain	Root, Stem bark	Fruit	0.60	Mucilage Pectins Catechins, Flavonoids, Vita A, Vita B3, Vita C, Vita K1, Vita E	Kini et al. (2008) and Makalao et al. (2015)

No known use, Ca: Calcium, Mg: Magnesium, P: Phosphorus, Na: Sodium, Al: Aluminum, Fe: Iron, K: Potassium, V: Vanadium, Pb: Lead Vita: Vitamin, Se: Selenium, I: Iodine, Mn: Manganese, Cu: Copper, Cr: Chromium.

that tannins, flavonoids and flavonols are able to inhibit lipoxygenase L-1 and cyclooxygenase-1, two enzymes involved in the production of inflammation mediators (Allcarz and Jimenez, 1988; Ayo et al., 2013). This could justify the use of certain plants listed which contain them in the treatment of edema, fever or pelvic pain. Also, the astringent and healing properties of tannins (Derbré and Lamassiaude-Peyramaure, 2010) would justify the use of some plants that contain them in the treatment of many skin diseases during menopause. Alkaloids are compounds known for their action on the central nervous system and their calming effect. This could justify the use of certain plants that contain them such as *Daniella olivieri, Feretia apodanthera, Leptadenia hastata, P. biglobosa, Sarcocephalus latifoluis, Vitex doniana* in the treatment of sleep disorders and anxiety during the menopause period (Table 1). Many species listed (60.72%) were food plants well known by population with high use values (UVs ≥ 0.50). Indeed, various mineral elements, vitamins, proteins, lipids, carbohydrates, gum, resin, fibers are present in these plants as showed

Ficus sycomorus L.

Combretum glutinosum Perr.Ex DC

(a)

Gardenia soketensis Hutch

Acacia gourmaensis A. Rich.

(b)

Antada africana Guill.&Perr

Psidium guajava L.

(c)

Vitex doniana L.

Sarcocephalus latifoluis (Sm.) E.A.Bruce

(d)

Securidaca longepedunculata Fresen.

Feretia apodanthera Del.

(e)

Figure 1. Plants used in the treatment of the most common menopausal symptoms - a) hot flashes b) itching c) lower abdominal pain d) insomnia and anxiety and e) Edema.
Source: (Photos) Ramde-Tiendrebeogo Alphonsine and Hien Sabine, 2018

by previous work (Table 1). Women must therefore be made aware of the menopause period in order to encourage them to choose foods – health whose effectiveness has been proven by previous studies. It is also urgent to develop and increase the range of natural nutraceuticals from the local plants of this study for the management of menopausal symptoms in women

Conclusion

The objective of this study was to make known the plants used in the treatment of the different symptoms of menopause by women in Burkina Faso. Results showed that local food plants are the most used. This constitutes a scientific database that could help women better manage the menopause period through a healthy and varied diet. These results also constitute a scientific support for the development of new natural nutraceuticals or improved traditional medicines for the treatment of menopausal disorders in women.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of *Withania somnifera* in the treatment of male infertility: A literature review

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The high prevalence of male infertility associated with the high costs of its conventional treatment motivated researchers to look for alternative approaches to the treatment of sexual dysfunction. The use of medicinal plants for these fins has been adopted in *Withania somnifera* which is one of the plants that has been associated with improvements in male fertility. Thus, the aim of this study was to review data available in the literature on the effect of *W. somnifera* in the treatment of male infertility. The Pubmed, Lilacs and Medline databases from 2009 to 2019 were searched and 9 articles selected, including intervention articles, systematic review and meta-analysis. Also known as Aswagandha or "Indian Ginseng", the part of *W. somnifera* most used in treating infertility is the root, mainly in the form of extract. It acts through two mechanisms: "oxidative" and "non-oxidative", both associated with improved sperm count, motility and morphology. However, further studies should be conducted investigating isolated phytochemicals to verify their true functionality.

Key words: Male infertility, male sexual disorders, *Withania somnifera*.

INTRODUCTION

Infertility means the inability to conceive after one year of unprotected sexual intercourse. Male factor infertility is the cause of 40-50% of cases and has become a significant health problem (Ayaz et al., 2018). Diagnosis occurs when seminal parameters such as sperm concentration, motility and morphology are abnormal. In these cases, it is recommended to start medical treatment (Duca et al., 2019; Vander Borght and Wyns, 2018).

Current treatments for male infertility, such as assisted reproduction techniques, have high costs and often low successful rates (10-30%) (Nejatbakhsh et al., 2016). Although hormonal (FSH-follicle replacement) and nutritional therapies (with carnitine, arginine, zinc, selenium, vitamin E, glutathione and coenzyme Q10) may be used to treat semen alterations (Duca et al., 2019), a new modality of treatment has contributed to improving the management of this issue (Nejatbakhsh et al., 2016). Alternative medicine, essentially herbal, has been projecting with the purpose of improving sperm parameters in male infertility (Sengupta et al., 2018).

The use of medicinal herbs has persisted for thousands

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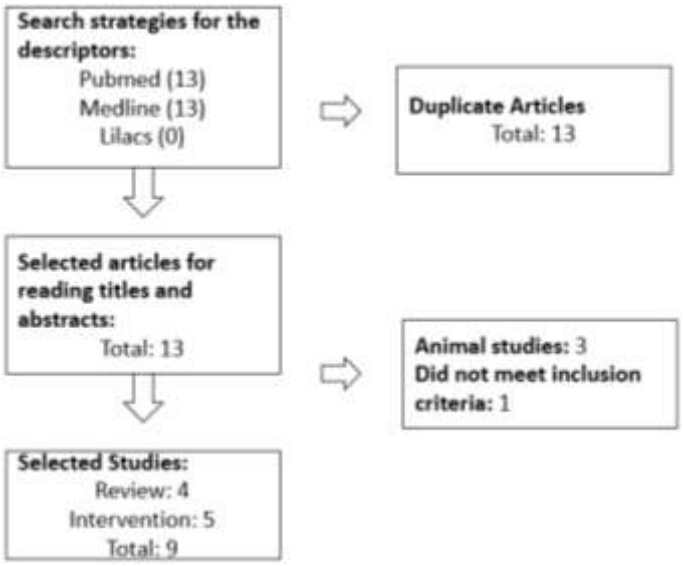

Figure 1. Flowchart of the articles search in the scientific literature.

years and continues to represent a considerable area of medical treatment in many countries, particularly in Asia, Africa and South America (Tahvilzadeh et al., 2016). According to an estimate by the World Health Organization (WHO), 80% of the world's population trust on herbal medicines for their care (Gadelha et al., 2015).

Essentially, the effect of medicinal plants on male reproductive function is associated with their antioxidant activity, since they present in their compositions phytochemical compounds able to inhibit spermatic membrane lipid peroxidation. Among these compounds are polyphenols, flavonoids and tannins, which act in various processes of the male reproductive system, such as spermatogenesis and steroidogenesis (Ahmadi et al., 2016; Lohiya et al., 2016; Mansouri et al., 2016). *Withania somnifera*, locally known as Aswagandha or "Indian Ginseng" is an Indian-origin herb that has been widely used in Brazil for its adaptogenic potential (Lohiya et al., 2016). Although commonly used in its countries of source as part of the treatment of erectile dysfunction, oligozoospermia, endocrinological reproductive problems and other male reproductive problems (Gupta et al., 2013; Kumar et al., 2015), in Brazil, it is still rarely used as complementary treatment for male infertility. Knowing that medicinal plants are recognized as a safe, effective and economical alternative and considering that *W. somnifera* has been used in the treatment of reproductive disorders, the aim of this study was to review the available data in literature on its effectiveness in improving male infertility.

METHODS

A literature review was performed using three databases: Pubmed, Lilacs and Medline, in order to identify scientific articles published from 2009 to 2019. Search was performed based on the descriptors "Male Infertility" or "Infertile Men" to describe the population, *"W. somnifera*" "Aswagandha" or "Indian Ginseng" to describe the intervention, and "Sperm Parameters" "Sexual Dysfunctions or "Men's Sexual Health" for the outcome.

Human intervention studies were included, as well as systematic reviews and meta-analyzes. Male reproductive disorders with the following diagnoses were included: Oligozoospermia (reduced sperm count in semen), asthenozoospermia (decreased sperm motility), azoospermia (absence of sperm), oligoastenozoospermia (low sperm concentration and motility) or teratozoospermia (altered sperm morphology). Animal studies were not included.

The publications were pre-selected by titles, which had to include the full terms and/or references to male infertility and *W. somnifera;* thereafter the abstracts were read. Articles that met the inclusion criteria entered the study, otherwise, they were excluded. After searching, Endnote X8 was used to remove duplicate articles.

The flowchart (Figure 1) describes the study selection process. Finally, 9 articles were selected for discussion.

RESULTS

General features

Found in hot, dry regions of the semi-arid climate, *W. somnifera* originates from South Africa, the Middle East, India, and China (Dar et al., 2015). In India, *W. somnifera* is a medicinal plant and both the whole plant and its parts are used in medicinal treatments. From ancient times, *W. somnifera* root has been used as adaptogen, diuretic, sedative, antioxidant and aphrodisiac (Narinderpal et al., 2013). Other parts of the plant, such as leaves and fruits, have been used as an analgesic, memory stimulant, anti-neoplastic, antimicrobial and anti-inflammatory agent (Sengupta et al., 2018).

W. somnifera contains a wide variety of phytochemicals

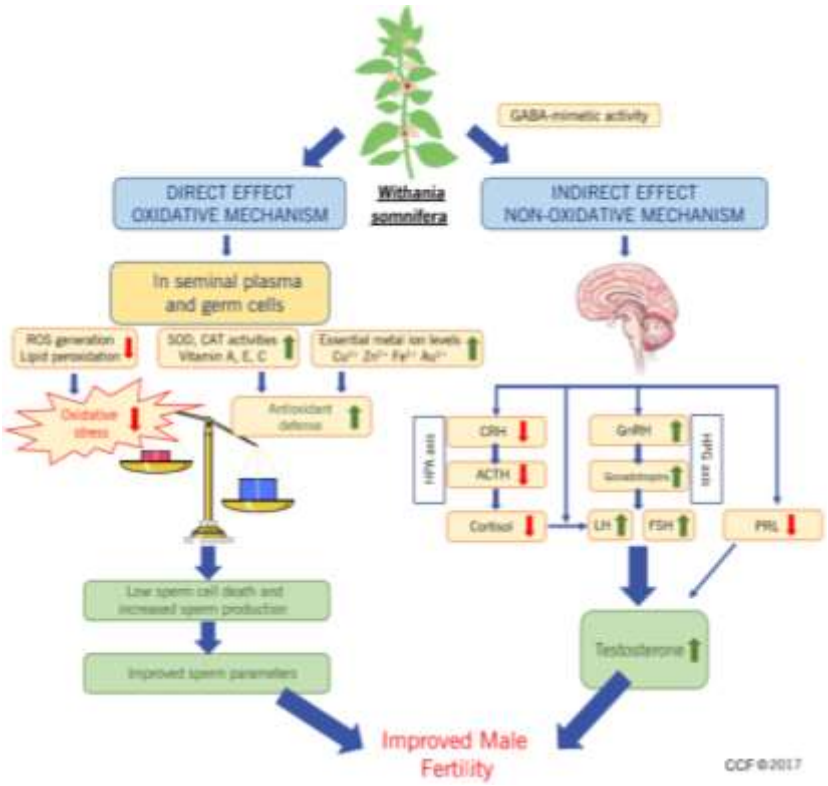

Figure 2. The proposed mechanism of action of *Withania somnifera*. Green arrows indicate an increase, while red arrows indicate a decrease. ACTH: Adrenocorticotropic hormone; CAT: catalase; CRH: corticotropin-releasing hormone: GABA: gamma-aminobutyric acid; GnRH: Gonadotropin releasing hormone; PAH: hypothalamus-pituitary-adrenal; HPG: hypothalamus-pituitary-gonadal; ROS: reactive oxygen species; SOD: superoxide dismutase; PRL: prolactin; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone.
Source: Adapted from Segpunta et al. (2018).

giving rise to a range of biological implications. In preclinical studies, it has demonstrated antimicrobial, anti-inflammatory, anti-tumor, anti-stress, neuroprotective, cardioprotective and antidiabetic properties (Dar et al., 2015).

In addition, this plant is known for its potential to promote health and longevity by preventing the aging process, enhancing defense against disease, revitalizing the body in debilitated conditions, enhancing an individual's ability to resist environmental effects and creating a sense of mental well-being (Durg et al., 2015).

Due to its pronounced anti-stress qualities, this species of plant is named "somnifera", which means "sleep inducer". The pharmacological effects and folk uses of *W. somnifera* are like those of Korean ginseng tea. For this reason, it is also known as Indian ginseng (Dar et al., 2015).

W. somnifera extract is one of the widely used herbal medicines for the treatment of infertility and sexual dysfunction. It contains more than 80 types of phytochemicals, among which are alkaloids and flavonoids. Its root is the most used part for certain purposes, as it has antioxidant, anti-apoptosis and anti-inflammatory properties and exerts positive effects on the male reproductive system, improving semen quality by regulating sex hormone levels and inhibiting lipid peroxidation (Azgomi et al., 2015).

Mechanisms of action

The exact mechanisms underlying the reproductive effects of *W. somnifera* are not yet well elucidated however, several studies have shown that its phytochemical components may exert major effects on the male reproductive system through antioxidant and detoxicant properties, regulation of sex hormones, GABA-mimetic action (Tahvilzadeh et al., 2016; Kumar et al., 2015; Ambiye et al., 2013). The mechanisms by which *W. somnifera* is capable of exerting effects on the male reproductive system and fertility can be divided into oxidative and non-oxidative mechanisms as shown in Figure 2 (Sengupta et al., 2018; Mahdi et al., 2011).

DISCUSSION

Oxidative mechanism

First, it is important to note that reactive oxygen species cannot be only harmful as several biological reactions at the chemical level are by then catalyzed. From fertility point of view, an optimal concentration of reactive oxygen species (ROS) is required for sperm maturation, capacitation, hyperactivation, acrosome reaction, zona pellucida binding and sperm-oocyte interaction (Agarwal et al., 2014).

However, excessive production of reactive oxygen species (ROS) may be among the main causes for the onset of male reproductive dysfunction, as the sperm membrane presents high amounts of polyunsaturated fatty acids, making them susceptible to lipid peroxidation (LPO) (Bisht and Dada, 2017). A high concentration of ROS or low antioxidant intake can lead to the process known as oxidative stress, resulting in the disruption of sperm cell DNA and/or RNA molecules (Esmaeili et al., 2015). Thus, antioxidants in seminal plasma protect germ cells against such damage and prevent the formation of ROS (Aitken, 2016).

The antioxidant action of *W. somnifera* has been determined by the amount of LPO produced. It has been observed that compounds extracted from *W. somnifera* are able to donate electrons and stop the destructive chain reaction of free radicals, thus decreasing the general charge of ROS (Gadelha et al., 2015). *Withania* flavonoids have been reported to have potent antioxidant activity that can counteract the formation of ROS in infertile men. Two alkaloids, sitoindosides VII-X and withaferin A, have been shown to activate the major free radical scavenging enzymes in vivo (Ahmad et al., 2010).

Metal ions, such as Cu^{2+}, Zn^{2+} and Fe^{2+} are cofactors for the antioxidant enzymes superoxide dismutase (SOD) and catalase and their deficiency has been observed in the sperm of infertile men (Shukla et al., 2011). Treatment with *W. somnifera* significantly improved SOD activity, catalase activity and glutathione level, eventually reducing protein peroxidation and carbonyl levels in infertile men (Durg et al., 2015).

W. somnifera root has also been shown to increase alanine concentration in semen. Alanine has a protective role against oxidative stress and can reduce LPO and thus increase sperm concentration and motility (Tahvilzadeh et al., 2016; Gupta et al., 2013).

Thus, the ability of *W. somnifera* root extract to decrease ROS can be inferred from two factors: its potent antioxidant activity and its ability to improve the concentration of metal ions that act as enzymatic cofactors (Shukla et al., 2011).

Non-oxidative mechanism

In addition to oxidative stress, other causes, such as hormonal imbalance caused by physiological or psychological factors, are also related to male infertility. Stress-related hormones, particularly glucocorticoids, have a deleterious effect on the hypothalamus-pituitary-gonadal axis (HPG) and subsequently on spermatogenesis (Chandra et al., 2012). The hypothalamus gonadotropin-releasing hormone (GnRH) stimulates the anterior pituitary to release the Stimulating Hormone Follicle (FSH) and the Luteinizing Homonium (LH); both subsequently act on the gonads, regulating spermatogenesis and testosterone production. Therefore, when the HPG axis is disrupted by hormones such as gonadotropin inhibitor hormone, prolactin (PRL) and cortisol, spermatogenesis is negatively affected (Nargund, 2015).

Furthermore, stress increases production and release of epinephrine, norepinephrine and cortisol. These hormones can have a detrimental effect on male reproductive function with two main mechanisms altering local blood flow and causing vasoconstriction in target tissues; reducing LH functional activity and testosterone level, which leads to decreased libido and oligospermatogenesis (Mahdi et al., 2011).

Due to its adaptogenic effects, *W. somnifera* can promote homeostasis, reducing stress response and normalizing cortisol levels. These characteristics were confirmed in a study that analyzed its effects on infertile men with normozoospermia who were under psychological or environmental stress (smoking) or had infertility of unknown cause (Sengupta et al., 2018).

Ambiye et al. (2013) noted that treatment with Ashwagandha root extract resulted in higher levels of testosterone and LH among infertile men, which had suboptimal values prior to therapy. Thus, they postulated that the probable reason for increased sperm concentration and motility was the higher testosterone levels provided by treatment with the extract.

In addition to these mechanisms of action, the roots of *W. somnifera* contain substantial amounts of lactate and lactate dehydrogenase (LDH). The stimulation of Krebs cycle and the consequent increase in ATP and cAMP levels observed after 3 months of treatment could help explain the achieved improvement in sperm concentration and motility (Tahvilzadeh et al., 2016).

Based on the intervention studies already performed the Table 1 was elaborated upon which summarizes the administered treatments and the effects of *W. somnifera* on sperm quality. The main conclusions of the systematic review studies are summarized in Table 2.

Conclusion

In recent years, the modern medical sciences have prospered greatly in treating infertility. However, despite major advances in synthetic products, herbal products are still a preferred choice in terms of safety and accessibility.

Table 1. Characteristics and results of human studies investigating the effects of *W. somnifera* (Ws) on the reproductive system.

Objective	Model used in fertility status	Treatment	Effect on sperm quality	Study suggestion	Reference
1. Effect of *Ws* root powder on semen quality in infertile men with "under stress" normozoospermia	Infertile men categorized into three groups (N = 20 in each): i) heavy smokers; ii) under psychological stress iii) with infertility of unknown etiology. Control: Fertile Men (N = 60)	*Ws.* root 5 g / day for 3 months plus skimmed milk.	In infertile men, there was an increase in sperm concentration compared to pretreatment values ($p < 0.01$). In men under psychological stress, increased sperm concentration ($P<0.01$) and motility ($P < 0.05$). In male smokers, increased sperm concentration and motility ($P<0.05$, respectively).	Fractionate this herb to better understand the mechanism of action of each constituent.	Mahdi et al. (2009)
2. Effect of Ws root extract on semen quality in infertile men	Infertile men with Normozoospermia, oligozoospermia and asthenozoospermia (n = 25 for each group)	5 g / day orally for 3 months with a cup of milk	Semen volume increased in men with normozoospermia and oligozoospermia compared to pretreatment values ($P<0.01$). Sperm concentration, motility and sperm count increased significantly in men in the three groups ($P < 0.01$).	Further study is needed to explore the individual properties of active constituents.	Ahmad et al. (2010)
3. The effect of Ws administration on apoptosis and ROS and metal ion concentrations in infertile individuals	Infertile men with Normozoospermia, oligozoospermia or asthenozoospermia (n = 25 for each classification) Control: Fertile men (N = 75)	5 g / day of single dose Ws root powder with milk for a period of 3 months for both groups	Significant reduction of apoptosis in normospermic and oligospermic men and ROS concentrations in oligozoospermic and astenozoospermic men (all $p<0.05$). Treatment also significantly improved overall metal ion concentrations ($p<0.01$).	More studies with larger sample size and placebo control group	Shukla et al. (2011)
4.Effect of Ws root extract on spermatogenic activity	46 male patients with oligospermia were included and randomized to treatment with extract (n = 21) or placebo (n = 25) on the same protocol.	675 mg / day in three doses for 90 days	There was a 167% increase in sperm count ($p<0.0001$), a 53% increase in semen volume ($p<0.0001$) and a 57% increase in sperm count ($p<0.0001$) among those who received sperm treatment. In addition, a significant and regular improvement was observed in serum hormone levels with WS treatment compared with placebo.	Suggests further exploration.	Ambiye et al. (2013)
5. Comparison of the effects of Ws with pentoxifylline on sperm parameters in idiopathic male infertility	Infertile men (N = 100) were randomly allocated to groups to receive Ws or pentoxifylline.	50 participants received Ws root in 6 capsules produced in two different colors (containing 5 g of Ws root) and 50 participants received pentoxifylline in 6 capsules in two different colors (containing 800 mg of this drug and a placebo) three times a day for 90 days.	*W. somnifera* increased mean sperm count (12.5%) and progressive motility (21.42%) and improved sperm morphology as an alternative to pentoxifylline	Large-scale studies with longer follow-up could be beneficial in elucidating further details of the efficacy and effectiveness of Ws in idiopathic male infertility.	Azgomi et al. (2018)

As discussed, *W. somnifera* contains several active constituents. Although the exact mechanism of action still needs further exploration, its main effects should be the reduction of oxidative stress, the regulation of hormone levels and the improvement of detoxification processes in the body.

Due to the growing interest in the use of herbal medicines, especially those with antioxidant and reproductive support properties, it is necessary to expand the number of studies in the area, encompassing a larger population and more population and more structured methodology. This could lead to more accurate conclusions and to an establishment or change of conduct in

Table 2. Characteristics and results of review and meta-analyzes investigating the effects of *W. somnifera* (Ws) on the reproductive system.

Objective	Discussion	Study suggestion	Reference
1. Evidence based on the application of medicinal plants in the treatment of sperm abnormalities in Persian Traditional Medicine	Most herbs introduced in this study have been clinically investigated for their effects on semen parameters, including sperm count and motility, sperm viability, dead or abnormal sperm, and recovery of sperm morphology. For *Ws*, more reliable evidence was found.	Overall, studies on the effectiveness of herbal remedies proposed for male infertility are promising; However, further studies are recommended for more conclusive results in the efficacy and safety of medicinal plants.	Tahvilzade et al. (2016)
2. Role of *Ws* (Ashwagandha) in the management of male infertility	Molecular mechanisms of action have been provided to better understand how *Ws* exerts its effects.	This review is unable to declare *Ws* as a "safe" or "toxic" drug. However, from the results analyzed, it can be assumed that *Ws* is beneficial for male fertility in several respects.	Segpunta et al. (2018)
3. Effect of *Ws* on the Reproductive System: A Systematic Review of Available Evidence	Ws exerts antioxidant properties, improves semen parameters and regulates sex hormones. These effects can affect many men seeking infertility treatment as they have a lower cost benefit and do not appear to have an adverse side effect. When associated with lifestyle modification and standard medical treatment, the chances of pregnancy are increased.	Further studies with a larger population and a more structured method are proposed so that a more accurate analysis can be made.	Azgomi et al. (2018)
4. Pharmacological overview of *Ws*, Indian Ginseng	Ws is a natural product with promising pharmacological and pharmaceutical properties. it or its constituents exert multiple protective properties, such as anti-inflammatory, antioxidant, inhibiting NFK-b transcription, MAPK signaling pathways, antiapoptotic, angiogenic and stress-reducing effects.	Further clinical validation needs to be performed for your general medical use.	Dar et al. (2015)

clinical practice.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Evaluation of aphrodisiac properties of the aqueous extract of the trunk barks of *Spathodea campanulata* P. Beauv. (Bignoniaceae) on albino rats (*Rattus norvegicus*)

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Spathodea campanulata P. Beauvois (Bignoniaceae) is a tree from the tropical and subtropical forests of Africa, used in folk medicine for the treatment of several diseases such as gastric pain, rheumatism, lumbago, cataracts and some intestinal parasites. In West Cameroon, traditional healers use a decoction of the bark of the trunk as an aphrodisiac in males. The objective of the present study was to evaluate the aphrodisiac activity of the aqueous extract of the trunk barks of S. campanulata in male rats. The male rats were divided into five lots: A, B, C, D and E of six animals each. Lot A received 5 ml/kg of distilled water daily for 8 days (negative control). Lot B received 5 mg/kg of Sildenafil Citrate (Viagra®) daily for 8 days (positive control). Lots C, D and E received 200, 400 and 800 mg/kg, respectively of the aqueous extract of the trunk barks of S. campanulata daily for 8 days. On the first, fourth and eighth day of administration, the copulatory parameters were observed and recorded. The extract induced an increase in erectile function stimulation through the significant increase ($p<0.001$) in the number of erections, the frequency of mount and a decrease in mount latency, reflecting an increase in sexual stimulation; an increase in the frequency of intromission ($p<0.001$) and a decrease in intromission latency, reflecting a stimulation of sexual performance. There was also an increase in ejaculation frequency and ejaculation latency ($p<0.001$). These results indicate a pro-ejaculatory aphrodisiac potential of the aqueous extract of the trunk barks of S. campanulata in male rats and would justify the empirical use of this plant in the treatment of erectile dysfunction in humans, in traditional medicine.

Key words: Spathodea campanulata, aqueous extract, erectile dysfunction, aphrodisiac, male rat.

INTRODUCTION

Erectile dysfunction (ED) commonly known as sexual impotence is an inability to achieve or maintain sufficient

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penile erection to maintain full and satisfying sexual intercourse for at least six months (NIH Consensus Development Panel on Impotence, 1993). In 1995, about 152 million people worldwide were affected by ED and these numbers could reach 325 million by 2025 (Meuleman, 2003; Oladiji et al., 2013). In 2012, the global prevalence of ED was estimated at 20%, with a proportion increasing sharply with age (Andersson and Wagner, 1995). In the population aged 40 to 70, the incidence of erectile dysfunction is approximately 50%. Among men aged 18 to 69 who are sexually active, 47% reported at least an occasional erectile dysfunction and 7% persistent erectile dysfunction (VIDAL-Erectile Dysfunction-The disease, 2018). It appears that erectile dysfunction (ED) represents a real public health problem that profoundly affects the quality of life of patients and their partners (NIH Consensus Development Panel on Impotence, 1993; Elbendary et al., 2009). The causes of ED are organic, metabolic and/or psychological (ignorance of sexuality, anxiety, performance anxiety, family background and childhood, extramarital relationships or breakdown) (NIH Consensus Development Panel on Impotence, 1993). It is therefore crucial to improve the quality of sexual life of men who have it, as well as that of their partners, by providing adequate and effective treatment.

Thus, treatment options such as penile prostheses, psychosexual and oral therapies are implemented to remedy this problem (Porst, 2002; Meuleman, 2003). There has been considerable progress in research over the last few decades in the medical management of ED, and oral treatments are nowadays the most widely used, particularly in addition to the discovery of phosphodiesterase type 5 (PDE5) inhibitors such as Sildenafil, Tadalafil or Vardenafil, the use of α 2-adrenergic receptor antagonists such as Yohimbine or dopamine receptor agonists such as Apomorphine (Porst, 2002; Pryor, 2002; Fink et al., 2002; Drewes et al., 2003). The high cost of these conventional treatments as well as the poverty of the populations of African countries have aroused in recent years a growing interest and a strong demand for traditional herbal medicines. Several plants are then used by African populations as having aphrodisiac properties that is to say that they are known to be able to create or stimulate sexual desire (Tajuddin et al., 2004; Singh et al., 2013a). Some of these plants have been the subject of scientific research (Tajuddin et al., 2003; Watcho et al., 2006; El-Tantawy et al., 2007; Sanda et al., 2012) and others not researched on. This is the case of *Spathodea campanulata* P. Beauv. (Bignoniaceae) locally called "foukfouk" or "foufougue" which is used by traditional healers in the region of West-Cameroon (Roger et al., 2015) and West African regions for the treatment of ED (KondakuMbuta and Institute for Health Sciences Research, Forest Genetic Resources Program in Sub-Saharan Africa, 2012). Thus, it is essential to make a scientific contribution to their use.

This study was undertaken to evaluate the aphrodisiac properties of aqueous extracts of the trunk bark of *S. campanulata* (Bignoniaceae) in albino rats (*Rattus norvegicus*).

MATERIALS AND METHODS

Plant material

Harvest and identification

The bark of the trunk of *S. campanulata*, was harvested in November 2016 in the village of Foto district of Dschang, Department of Menoua in the region of West Cameroon. The identification of the plant was carried out at the national herbarium of Cameroon in comparison with the sample of *S. campanulata* collected by D. W. Thomas at the national herbarium under the number 55489 HNC/YA of the family Bignoniaceae. A certificate of identification has been issued for this purpose.

Preparation of extracts

Preparation of the aqueous extract of *S. campanulata*: The bark of the trunk of *S. campanulata* was sorted, cleaned and dried at room temperature away from the sun for ten days. They were then milled with an electric grinder and the resulting powder was subjected to aqueous extraction on one hand and to organic solvents by increasing polarity on the other hand: 3750 g of powder was macerated for 72 H in 5 L of water. The mixture was stirred every 24 h. After filtration with Whatman No. 3 paper, the macerate obtained was concentrated in an oven at 55°C. The aqueous extract obtained weighed 69.2 g (1.86%). The stock solutions of respective concentrations 11.5, 27 and 48 mg/ml were prepared by diluting the extract in distilled water and stored at 4°C for later use.

Phytochemical screening

The phytochemical screening of the aqueous extract of the trunk barks of S*. campanulata* was carried out in the phytochemistry laboratory of the Faculty of Medicine and Pharmaceutical Sciences (FMSP), according to the protocol described by Harborne (1980) slightly modified.

Animal material and ethical considerations

The animal species on which the present studies were conducted were rats (*Rattus norvegicus*) of the Wistar albino strain. The rats were raised in the pet shop of the Faculty of Medicine and Pharmaceutical Sciences (FMSP) at room temperature following the circadian rhythm. These animals received daily standard food and drinking water *ad libitum*. Rats were bred in cages with shavings that were changed every 2 days. For this study, we selected male and female sexually experienced Wistar albino rats, aged 12 to 16 weeks, weighing between 144 and 212 g. Ovariectomized females were induced artificially into estrus for our different experiments.

Induction of estrus

Females were induced into estrus by successive administration of estradiol benzoate (15 µg/kg) and progesterone (60 µg/kg), respectively for 48 and 6 h before the start of the experiment. This protocol was approved by the Institutional Ethics Committee of the University of Douala.

Table 1. Phytochemical screening of the bark of the trunk of *Spathodea campanulata*.

Test	Secondary metabolites	Solvent extracts			
		Hexane	Ethylacetate	Methanol	Aqueous
Dragendorff	Alkaloids	+	+	+	+
Libermann Bushard	Terpenes and sterols	-	-	-	-
Saponins	Saponosides	-	+	+	+
Acetate	Coumarin	-	-	-	-
Ferric Chlorure	Phenols	-	-	+	+
Shinoda	Flavonoids	-	-	+	+
	Tanins	-	-	+	+
Fehling Liquor	Reducing sugars	-	-	+	+
	Anthocyanins	-	-	-	-

+: Present; -: absent.

Treatments of experimental animals

Thirty vigorous males submitted successfully to the grip test were divided into 5 groups of 6 rats each and treated orally as follows: Lot A (negative control) received 5 mL/kg of distilled water daily for 8 days, Lot B (positive control) received 5 mg/kg of Sildenafil Citrate (Viagra®) daily for 8 days, Lots C, D and E received 200, 400 and 800 mg/kg aqueous extract of the trunk bark of *S. campanulata*, daily for 8 days. These substances were administered using an orogastric feeding tube.

Study protocol for male sexual behaviour

The study was conducted between 13:00 and 17:00 to avoid any potential interference with the increase in natural libido observed in the mornings. The observations were then made on the 1st, 4th and 8th day of treatments administration. Thus, each rat was placed in a cage for 1 h for acclimation, and then a receptive female was introduced into the cage. The pair of animals was observed closely for 30 min. During this time, the following copulation parameters were counted and latency times measured:

(1) The frequency of the mounts which corresponded to the number of mounts, with or without intromissions preceding an ejaculation;
(2) The number of erections;
(3) The frequency of intromissions which corresponded to the number of intromissions preceding an ejaculation;
(4) The frequency of ejaculations which is the number of ejaculations recorded during the observation time;
(5) The latency of the ride which is the time between the introduction of a female in the cage and the first mount;
(6) The intromission latency time which is the time between the introduction of the female and the first intromission;
(7) The latency time of ejaculation which is the time that separates the first intromission from the first ejaculation (Pfaus et al., 2016).

Statistical analysis

Data were entered into an Excel spreadsheet (Microsoft Office 2007, USA) and analyzed with Statview software version 5.0 (SAS Institute, Inc., USA). Quantitative data were presented as mean ± standard deviation (SD) in graphs and tables. One-way order of variance analysis was used to compare the averages between two and more than two groups, respectively. The Newman-Keuls post hoc test was used to make the multiple pair comparisons. The materiality threshold was set at p-value 0.05.

RESULTS

Phytochemical screening

The phytochemical screening of the aqueous extract of the trunk bark of *S. campanulata* revealed the presence of the secondary metabolites shown in Table 1.

Effects of the aqueous extract of *S. campanulata* on the mount frequency

The effects of the aqueous extract of *S. campanulata* on the frequency of mounts on days 1, 4 and 8 are shown in Table 1. On day 1, we observed a significant increase ($p < 0.05$) in the frequency of mounting rise for the batches treated at 200 and 400 mg/kg compared to the negative control. This increase was more marked for the lot at 400 mg/kg. On day 4 of experimentation, a significant increase ($p<0.001$) of the riding frequency was observed in all the animals treated with the extract compared to those treated with distilled water. At day 8, the riding frequency was significantly ($p < 0.05$) increased for lots (or batches) treated at 400 and 800 mg/kg of extract, compared to the control receiving distilled water (Table 2).

Effects of the aqueous extract of *S. campanulata* on the frequency of intromission

Table 2 shows the effects of the aqueous extract of *S. campanulata* on the frequency of intromission in the different groups of animals on days 1, 4 and 8 of the experimental period. On day 1, we observed a significant

Table 2. Effects of aqueous extract of *S. campanulata* on the mount frequency, on the intromission frequency, the number of erections the ejaculation frequency. All values were expressed as average + SD

Lots	Observed parameters (per 30 min)											
	FM			FI			NE			FE		
	J1	J4	J8	J1	J4	J8	J1	J4	J8	J1	J4	J8
Eau distillée	15 ± 0	13.00 ± 0	21 ± 0	18.83 ± 5.44	13.03 ± 3.63	5.32 ± 1.56	22.33 ± 3.64	19.83 ± 3.47	24.33 ± 3.04	0.83 ± 0.3	1.33 ± 0.42	1.33 ± 0.3
Citrate de Sildenafil	27.67 ± 5.95	31.50 ± 2.63	34.33 ± 1.99	25.00 ± 5.32	13.34 ± 2.17	5.45 ± 2.33	24.00 ± 6.11	25.17 ± 2.65	27.83 ± 2.69	1.17 ± 0.4	1.67 ± 0.33	2.17 ± 0.33
S. campanulata 200 mg/kg	23.33 ± 2.87	31.60 ± 1.99	31.17 ± 5.30	20.50 ± 2.51	6.16 ± 1.74	2.51 ± 5.00	22.50 ± 2.77	29.80 ± 2.15	27.50 ± 5.50	0.83 ± 0.17	1.6 ± 0.24a	1.33 ± 0.33
S. campanulata 400 mg/kg	29.00 ± 4.66	29.33 ± 1.36	36.33 ± 4.26	23.17 ± 4.22	10.34 ± 1.68	4.22 ± 3.95	27.50 ± 5.05	31.00 ± 2.58	36.17 ± 3.91	1.33 ± 0.33	2.67 ± 0.21	2.17 ± 0.17
S. campanulata 800 mg/kg	21.67 ± 2.64	32.67 ± 3.95	33.50 ± 4.98	18.83 ± 2.18	5.35 ± 3.61	2.18 ± 4.43	16.33 ± 1.41	32.67 ± 3.58	27.83 ± 5.13	0.67 ± 0.2	1.83 ± 0.3b	1.33 ± 0.42

FM: Mount frequency, FI: intromission frequency, NE: number of erections, FE: ejaculation frequency.

(p<0.05) increase in the frequency of intromission in the animals receiving 200 and 400 mg/kg extract compared to the negative control group. When we compared with the positive control, we observed that only the difference with the animals receiving 800 mg/kg of extract was significant (p<0.001). On day 4, we observed a significant decrease (p<0.001) of the intromission frequency for the lots treated with the extract at different doses compared to the negative control.

Effects of aqueous extract of *S. campanulata* on the number of erections

On day 1, we observed that the number of erections significantly increased (p<0.05) in animals receiving different doses of 200 and 400 mg/kg of the extract compared to the negative control. On day 4 of administration, we observed a higher significant increase (p<0.001) in the number of erections for the different lots treated with the extract compared to the negative control. The erection number for the positive control lot increased significantly every 3 days compared to the negative control (Table 2).

Effects of the aqueous extract of *S. campanulata* on the ejaculation frequency

On day 1, the frequency of ejaculations in animals receiving 200 and 400 mg/kg of extract was significantly increased (p<0.05), compared to that of the negative control group. The difference with the positive control is not significant. At day 4, we observed a significant (p<0.001) increase in ejaculation frequency for the 400 and 800 mg/kg treated groups compared to the negative control. The batch at 200 mg/kg showed a significant difference (p<0.05) compared to the same negative control. Also, we observed a significant increase (p<0.05), only for the batch treated with 400 mg/kg of extract compared to the positive control. On day 8, among the lots that received the extract, the lot receiving 400 mg/kg of extract was the only one that showed a significant difference (p<0.05) compared to the negative control group (Table 2).

Effects of aqueous extract of *S. campanulata* on mount latency

On day 1, we observed a significant decrease (p<0.001 and p<0.05) of latency in all animals receiving doses of 200, 400 and 800 mg/kg compared to the negative control group that received distilled water. On day 4, we observed a significant decrease (p<0.001) for the batches receiving 400 and 800 mg/kg of extract compared to the negative control. Compared to the positive control, we observed a significant increase in the latency of rats in rats receiving with 200 mg/kg of extract. On day 8, we observed a significant decrease in the latency of the batch treated at 200 and 400 mg/kg (Table 3).

Effects of the aqueous extract of *S. campanulata* on intromission latency

On day 1, we observed a significant decrease (p<0.05) in intromission latency for the batches receiving 200 and 800 mg/kg/day and a significant decrease (p<0.001) for the lot treated at 400 mg/kg compared to the negative control group receiving distilled water. Compared with the positive control lot, there was a significant increase (p<0.001) of the intromission latency time in rats given the 800 mg/kg extract. On day 4, we

Table 3. Effects of aqueous extract of *S. campanulata* on mounting latency, intromission latency, ejaculation latency. All values were expressed as average + SD.

Lots	Observed parameters (s)								
	TLM			TLI			TLE		
	J1	J4	J8	J1	J4	J8	J1	J4	J8
Eau distillée	71.33 ± 15.05	66.5 ± 2.06	91.5 ± 2.26	85.33 ± 5.11	75.50 ± 2.42	99.17± 9.74	725.67 ± 25.94	724.83 ± 26.94	602.00 ± 5.96
Citrate de Sildenafil	59.17 ± 22.82	58.83 ± 2.24	73.33 ± 3.56	59.67 ± 4.49	63.50 ± 3.2	81.33± 3.09	353.0 ± 57.7	304.50 ± 10.42	255.33 ± 7.54
S. campanulata 200 mg/kg	62 ± 11.07	75.17 ± 7.18	31.83 ± 3.61	82.00 ± 5.23	77.50 ± 1.77	34.83 ± 3.04	659.33 ± 33.51	639.83 ± 33.52	578.17 ± 2.81
S. campanulata 400 mg/kg	41.83 ± 9.42	18.17 ± 4.7	28.67 ± 1.18	50.50 ± 8.49	19.83 ± 4.6	28.67 ± 1.28	371.67 ± 45.87	351.50 ± 74.33	301.83 ± 7.3
S. campanulata 800 mg/kg	85.5 ± 19.05	32.67 ± 7.22	104.17 ± 4.48	87.00 ± 9.39	35.67 ± 7.99	106.00 ± 1.94	507.50 ± 15.49	496.50 ± 11.04	471.67 ± 10.01

TLM: Mounting latency, TLI: intromission latency, TLE: ejaculation latency.

observed a significant decrease in intromission latency time ($p<0.001$) for the lot receiving 800 mg/kg extract compared to the negative control. We also observed a significant decrease ($p<0.05$) of this same parameter for the batch receiving the extract at 400 mg/kg. On day 8, a significant decrease ($p<0.001$) in intromission latency was observed for lots (batches) receiving 200 and 400 mg/kg compared to the negative control (Table 3).

Effects of aqueous *S. campanulata* extract on ejaculation latency

The first day of the experiment showed a significant ($p<0.05$) increase in ejaculation latency in rats having received the dose of 200 mg/kg of the extract compared to those receiving distilled water. On day 8 of the experiment, the comparison with the negative control receiving distilled water showed a significant ($p <0.05$) increase in ejaculation latency in the animals treated with the extract at doses of 200 and 800 mg/kg (Table 3).

DISCUSSION

The overall objective of this study was to evaluate the aphrodisiac properties of the aqueous extract of the trunk barks of *S. campanulata* P. Beauv, a plant used in parts of sub-Saharan Africa and particularly in the West Region of Cameroon to treat erectile dysfunction. The findings of this study shows that high dose administration with aqueous extract of *S. campanulata* trunk bark stimulated the copulatory activity of treated rat lots, compared to the negative control group. Specifically, oral administration of the aqueous extract of *S. campanulata* to sexually experienced rats significantly increased the number of erections ($p<0.05$), frequency of mounts, intromissions and ejaculations for the lot received 400 mg/kg of aqueous extract of *S. campanulata* trunk bark. Latency mount and intromission time were reduced for the same batch, while ejaculation latency time significantly increased ($p<0.05$). Similar effects have been reported by Abedi et al. (2012) who evaluated the effects of the aqueous extract of pollen grains of *Phoenix dactylifera* on the sexual behavior of male rats. Thus, the extract would contain molecules that maintain erection and increase sexual motivation (Singh et al., 2013a). This is confirmed by the significant increase in the frequency of erections and ascents as well as the decrease in the latency of rats in the extract-treated rats compared to the negative control rats. On the other hand, the decrease in the latency period of ascending and intromission is an indicator of an aphrodisiac action (Yakubu et al., 2007). Indeed, the latency of riding and intromission are inversely proportional to the sexual motivation. Therefore, the significant decrease in elevation and intromission latency observed in rats receiving *S. campanulata* extract at 400 mg/kg on day 4 may suggest a stimulation of motivation and sexual arousal. These results go in the same direction as those obtained by Sanda et al. (2012) who also observed a marked increase in the frequency of erections, ascites and a decrease in the latency of mount in normal adult rats treated with aqueous extracts of *Allanblackia floribunda* and *Glycyrrhiza glabra*.

From these results, we can suggest an increase in motivation and sexual desire induced by the aqueous extract of *S. campanulata* trunk bark. This pro-sexual effect could be attributed to the existence of saponins (Drewes et al., 2003), flavonoids and alkaloids revealed in phytochemical studies of this plant. Indeed, the steroidal nature of saponins could act as an intermediary in the androgen production pathway. Saponins could also bind to steroid hormone receptors, which could lead to conformational changes and

contribute to an increase in the function of these hormones; or they could bind to the enzymes involved in testosterone synthesis and increase its production (Gauthaman and Ganesan, 2008). Saponins also have a peripheral action by stimulating the release of nitric oxide (NO) in vascular smooth muscle (Abedi et al*.,* 2012). The NO being a mediator involved in the relaxation of vascular smooth muscle tissue, will induce in erectile bodies of the penis, this increased vaso-relaxation is at the origin of an increase in the number of erections, hence the result that we got.

In addition, the reported antioxidant properties of *S. campanulata* (Mangambu et al*.*, 2014) and well-known flavonoids (reported as an elevator of androgen levels in animals) (Pelissero et al*.,* 1996) also contribute to the observed aphrodisiac effect. Similarly, the presence of alkaloids, known for their ergogenic properties, can act either by inducing vasodilatation of the blood vessels through NO production and ultimately leading to erection or by stimulating steroidogenesis in animal testes. Therefore, it is possible that the active ingredient contained in *S. campanulata* extract may have crossed the animal's blood-brain barrier to exert its aphrodisiac effect on the hypothalamic-hypophyso-testicular axis. It is well documented that in erectile function, androgens stimulate the expression of the neuronal isoform of nitric oxide synthase (nNOS) and modulate the activity of phosphodiesterase type 5 (Mills et al*.,* 1994). Alkaloids can also act by relaxing the smooth muscles of cavernous bodies in the copulatory organ of male rats. This aphrodisiac effect would be completely different from the action of Viagra® (sildenafil citrate), the reference molecule used in this study. Indeed, sildenafil citrate is a non-androgenic aphrodisiac that acts directly on penile erectile tissue inhibiting the activity of phosphodiesterase type 5, the enzyme involved in the specific degradation of cGMP, the second messenger involved in the mechanism of erection. The prolonged action of cGMP is at the base of the aphrodisiac effect of sildenafil citrate.

Conclusion

This study focused on the evaluation of the aphrodisiac activity of the aqueous extract of *S. campanulata* P. Beauv. We obtained the different extracts and characterized the large groups of secondary metabolites present in the extract. In addition, we tested the aphrodisiac activity compared to distilled water as a negative control on one hand and sildenafil citrate (Viagra®) on the other hand, which is a reference aphrodisiac sold in pharmacies as a positive control. The qualitative analysis of extracts of *S. campanulata* P. Beauv shows the presence of flavonoids, alkaloids, phenolic compounds, tannins, reducing compounds, saponins and coumarins. The study of the aphrodisiac activity shows that *S. campanulata* P. Beauv increases sexual desire in rats significantly compared to the negative control. Oral administration of the aqueous extract of the trunk barks of *S. campanulata* P. Beauv at doses of 200, 400 and 800 mg/kg body weight over a period of 8 days influenced the copulatory activity of normal and sexually experienced male Wistar albino rats. Compared with the animals in the negative control groups, the effects of the extract on the sexual behavior of the treated rats were materialized by stimulation of sexual motivation, effect confirmed by the increase in the frequencies of erections and mounts as well as the decrease in the latency of mount; stimulation of sexual performance, effect confirmed by the increase in the frequency of intromissions as well as the decrease of the latency of intromission; stimulation of sexual pleasure by an increase in the frequency of ejaculation and the latency of ejaculation, effect confirmed by the increase of the frequency of ejaculations and the increase of the latency of ejaculation (Singh et al., 2013b). It can be concluded that the aqueous extract of the trunk barks of *S. campanulata* P. Beauv have a pro-ejaculatory aphrodisiac effect. These results would justify the empirical use of this plant to fight against erectile dysfunction.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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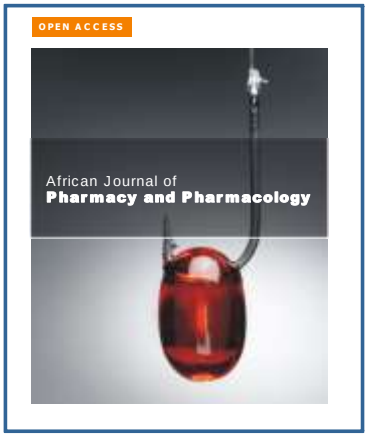

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