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Journal of Pharmacognosy and Phytotherapy

Full Length Research Paper

Acetyl and buteryl cholinesterase inhibitory effect of *Peltophorum pterocarpum* (DC) Backer ex K. Heyne (family Leguminosae)

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Accepted 1 March, 2013

Peltophorum pterocarpum, family Leguminosae is a tree natural to tropical South-Eastern Asia and was brought to Nigeria by immigrants. It has been used traditionally by the South Western people of the country as memory enhancer and anti-ageing. The present study was done to assess the cholinesterase inhibitory activity of the extracts and fractions of _P. pterocarpum_ using spectrophotometric and thin layer chromatography (TLC) bioautographic assay methods. Eserin was used as reference cholinesterase inhibitors. The methanolic extract of the leaves, root bark and stem bark were found to be active. The stem-bark gave the highest activity (68.85±3.53%) and better selectivity towards acetylcholinesterase (AChE) at a dose of 42.5 µg/ml followed by the root bark which inhibited both AChE and butyrylcholinesterase (BuChE) at 48.46±4.47 and 51.77±2.20, respectively and then the leaves with values of 47.50+2.41 and 48.91+0.71 against AChE and BuChE, respectively. Fractionation of the various plant parts showed that the active constituent many be moderately polar being mostly extractable in ethyl acetate and that purification leads to improved activity. These results demonstrate that _P. pterocarpum_ inhibits cholinesterase and thus may be relevant in the treatment of memory dysfunctions and neurodegenerative disorders such as Alzheimer's disease.

Key words: *Peltophorum pterocarpum*, acetylcholinesterase, butyrylcholinesterase, memory dysfunctions.

INTRODUCTION

Memory loss also referred to as amnesia is an abnormal degree of forgetfulness and/or inability to recall past events. In Nigeria, memory loss is considered a major problem in the traditional setting particularly among the youth who seek memory enhancing remedied to pass examinations. Plants have been used to treat memory related disorder for centuries (Perry et al., 2000). The use of complementary medicines such as plant extracts in dementia therapy however varies according to different cultural traditions (Perry et al., 1996). *Huperzia serata* and *Ginkgo biloba* have been used in Chinese medicine while *Salvia officinalis* and *Salvia lavandulaefolia* have been used in Europe (Grieve, 1980; Ryman, 1991; Tyler, 1993; Birks et al., 2004).

Inhibition of acetyl cholinesterase, the key enzyme in the breakdown of acetylcholine is considered a promising strategy in the treatment of neurological disorders such as Alzheimer's disease, senile dementia and myasthenia gravis (Blockland, 1996; Crisby et al., 2002). Many plants with a history of use as memory enhancer have been shown to contain cholinesterase inhibitors. Naturally occurring cholinesterases inhibitors continue to be identified in a wide variety of plant species (Tang, 1994; Park et al., 1996). *Peltophorum pterocarpum* (DC)

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Backer ex K. Heyne. (Leguminosae) is commonly referred to as golden flamboyant. It is a wonderful shade tree for a large landscape especially when in full bloom. It is used as astringent to cure or relieve intestinal disorder, after pain at child birth, sprains, bruises and swelling. It is also used as lotion for eye troubles, muscular pains and sores (Sethuraman et al., 1984). It is used as gargles and tooth powders (Sethuraman et al., 1984). Satish et al. (2007) reported the antifungal activity of *P. pterocarpum* against seed borne pathogens of *Aspergillus* species. Sethuraman et al. (1984) reported the anti-inflammatory and antibacterial activities of the flower. The antimicrobial activity was also reported by Duraipandiyan et al. (2006). Although, certain biological activity of *P. pterocarpum* has been reported, its memory enhancing potential has not received due attention. This study therefore explores the potential of *P. pterocarpum* as a cholinesterase inhibitor with a view to examining its folkloric claim as memory enhancer.

MATERIALS AND METHODS

Preparation of plant extract

Fresh sample of the various parts (leave, fruits, stem bark and root bark) of *P. pterocarpum* were collected from Road 7, Obafemi Awolowo University Campus, Ile-Ife, Osun State, Nigeria after proper identification by Mr. T. A Oladele of the Department of Pharmacognosy, Faculty of Pharmacy and authentication by Dr. H. Illoh of Botany Department, Obafemi Awolowo University where voucher specimen was deposited.

Collected samples were macerated with 80% methanol for 72 h. The extracts were concentrated *in vacuo* to dryness at 40°C.

Preliminary phytochemical screening

Preliminary phytochemical analysis of the extracts was carried out using standard methods (Harborne, 1992; Abulude, 2007; Kokate, 1994).

Fractionation of the methanolic extracts

The methanolic extracts of the various parts were partitioned into n-hexane, ethyl acetate and water. The various fractions were concentrated *in vacuo* at 40°C and were tested.

Vacuum liquid chromatography (VLC)

The most active ethyl acetate fraction was subjected to VLC on silica gel using n-hexane, ethyl acetate and water in various ratio as solvent system. Five subfractions were obtained based on thin layer chromatography (TLC) pattern. These subfractions were tested for acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activity.

Cholinesterase inhibitory assay

AChE and BuChE inhibitions were determined spectrophotometrically using acetyl thiocholine iodide (ATCI) and buterylcholine chloride (BTCI) as substrate, respectively by the modified method of Ellman (Ellman et al., 1961).

The reaction assay mixture consisted of 2000 ml 100 mM phosphate buffer, pH 8.0, 100 m of test sample stock solution in methanol (at a final concentration of 42.5 µg/ml), 100 ml of enzyme AchE or BuChE solution at a final concentration of 0.003 and 0.001 µg/ml, respectively. 100 µl of 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB) (0.3 mM) prepared in 100 M phosphate buffer, pH 7.0 containing 120 mM sodium bicarbonate. The reaction mixture was vortexed and then pre-incubated in a water bath at 37°C for 30 min. The reaction was initiated by the addition of 100 µl of ATCI or BTCI at a final concentration of 0.5 mM. As a negative control, the inhibitor solution was replaced with methanol. The change in absorbance at λmax 412 was then measured for a period of 5 min at room temperature. All assays were carried out in triplicate. Eserin ((-) physostigmine) was used as positive control. The percentage inhibition was calculated as follows:

$$\text{Percentage} = \frac{a-b}{a} \times 100$$

where a = ΔA/min of control; b = ΔA/min of test sample; ΔA = change in absorbance.

The crude methanolic extract, the various fractions, and subfractions were subjected to this test.

Table 1. Cholinesterase inhibitory activity of methanolic extract of different plant parts (AChE and BuChE).

Plant part	AChE inhibition (%)	BuChE inhibition (%)
Leaves	47.50 ± 2.41	48.9 ± 0.71
Root bark	48.46 ± 4.47	51.77 ± 2.20
Stem bark	68.85 ± 3.53	3.05 ± 0.58
Fruits	15.68 ± 1.37	4.31 ± 0.22
Eserin	90.31 ± 3.55	84.27 ± 4.72

RESULTS

Phytochemical screening

The preliminary qualitative phytochemical screening of *P. pterocarpum* revealed the presence of flavonoids, alkaloids and saponins in all the plant parts. Cardiac glycosides was present in the leaves, stem bark and root bark while tannins was positive only in the stem bark and root bark.

Cholinesterase inhibition of methanolic extract

The methanolic extract of *P. pterocarpum* showed inhibitory activity against AChE for all the different plant parts except the fruits as reported in Table 1. However, only the leaves and the root bark showed significant inhibition of BuChE.

Table 2. Cholinesterase inhibitory activity of different fractions (AChE/BuChE).

Fraction	AChE inhibition (%)	BuChE inhibition (%)
Leaves		
N	28.62 ± 1.37	22.01 ± 0.62
E	66.10 ± 0.78	46.32 ± 0.61
Aq	18.00 ± 0.99	19.23 ± 0.86
Stem bark		
N	26.66 ± 1.91	14.44 ± 0.96
E	70.10 ± 0.54	63.84 ± 0.67
Aq	29.14 ± 0.66	21.74 ± 0.39
Fruits		
N	10.90 ± 0.39	12.63 ± 0.57
E	40.58 ± 0.75	22.69 ± 0.68
Aq	31.07 ± 0.52	38.08 ± 0.80
Root bark		
N	34.02 ± 0.93	20.63 ± 0.49
E	69.91 ± 0.91	70.13 ± 0.73
Aq	13.28 ± 0.57	18.15 ± 0.88
Eserin	92.63 ± 1.98	89.30 ± 1.76

N: n-Hexane fractions; E: ethyl acetate fraction; Aq: Aqueous fraction.

Cholinesterase inhibition of various fractions

Cholinesterase inhibition was highest for the ethyl acetate fractions of all the different plant parts. This inhibition was generally on both AChE and BuChE (Table 2).

Cholinesterase inhibition of various subfractions

Five subfractions obtained from the VLC of the most active ethyl acetate fraction were also subjected to cholinesterase assay. The results are as shown in Figures 1 to 5).

DISCUSSION

Memory loss is a common feature in all cultures and plants have always been used for their management. In Chinese culture, the use of such plants as *Ginkgo biloba* which is an effective cholinesterase inhibitor, as memory enhancer has been justified (Atta-ur-Raham et al., 2001) and spectrophotometric assay method has been previously used by several authors to determine the cholinesterase inhibitory activity of many plants (Ellman et al., 1961, Houghton et al., 2004, Oh et al., 2004; Mukherjee et al., 2007).

The study was carried out to evaluate the acetyl and buteryl cholinesterase inhibitory activity of extracts of different plant parts (leaves, root bark, stem bark and fruits) and fractions of leaves of *P. pterocarpum.* Eserin (+ physostigmine) was used as reference standards. The study showed that methanolic extract of the fruits had no inhibitory effect on both AChE and BuChE. However, the extract of the leaves and the root bark inhibited both enzymes, while that of the stem bark selectively inhibited AChE. This result supports the claimed ethnomedical use of *P. pterocarpum* as memory enhancer since an inhibition of the enzymes will lead to an increase in the level of acetylcholine in the brain and hence better cognitive ability.

The plant parts were partitioned into n-hexane, ethyl acetate and water. The various partitioned fractions were also tested. It was observed that the ethyl acetate fraction of the various plant parts were most active. This suggests that the active principle is likely to be moderately polar.

The ethyl acetate fraction of the leaves, being the most regenerative part of the plant, was subjected to VLC and five subfractions (A to E) were obtained which were again tested. The effects of these subfractions at the various concentrations were time dependent. Highest inhibition was obtained within the first 30 s of the reaction. However, as the time increased, the activity of the fractions decreased. This observation was obtained

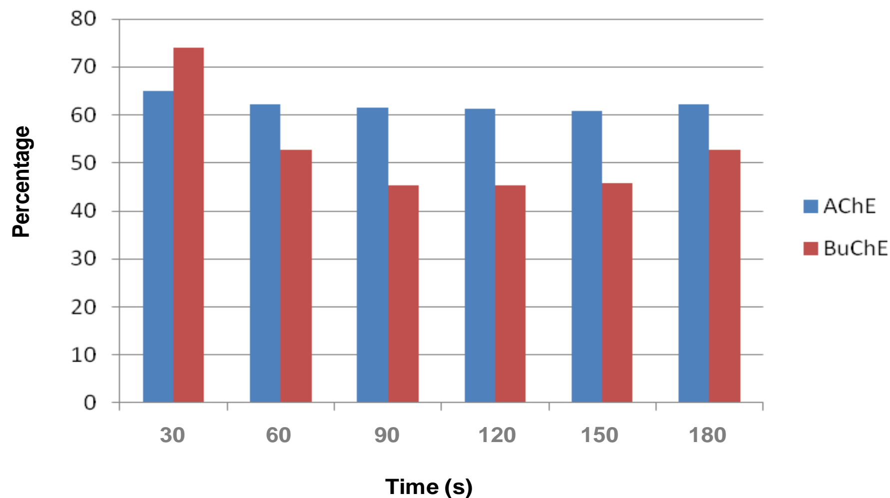

Figure 1. Percentage inhibition of subfraction A.

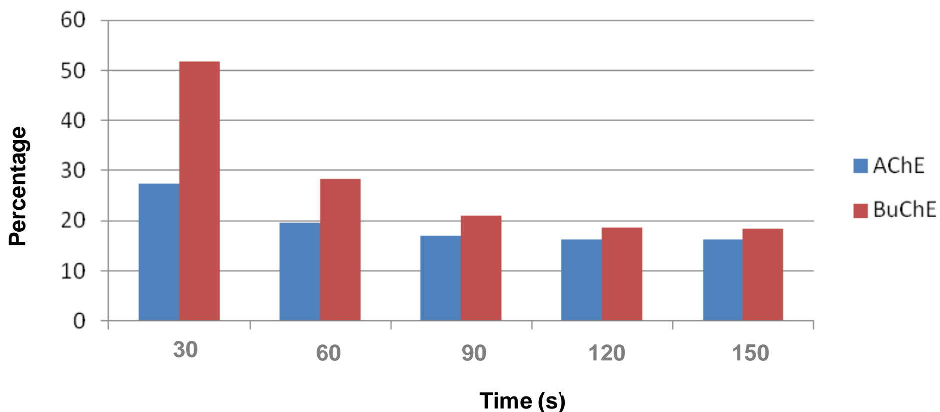

Figure 2. Percentage inhibition of subfraction B.

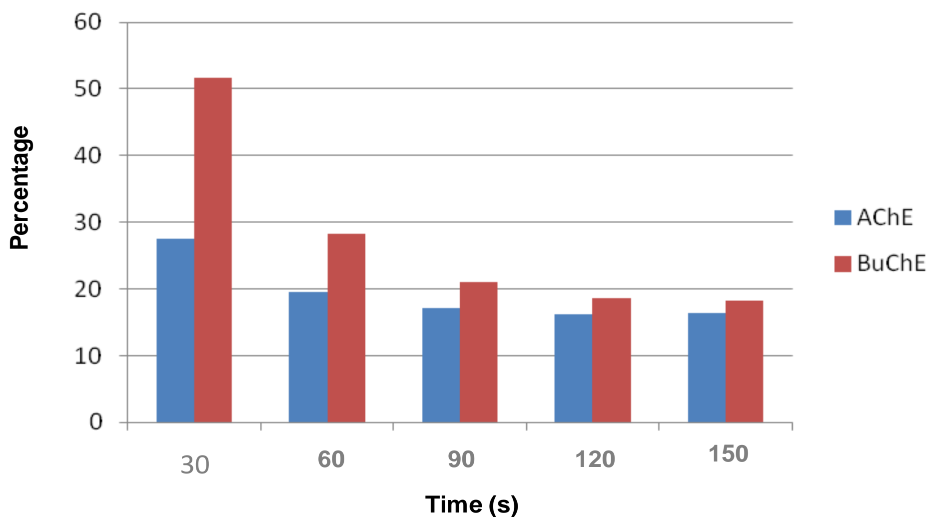

Figure 3. Percentage inhibition of subfraction C.

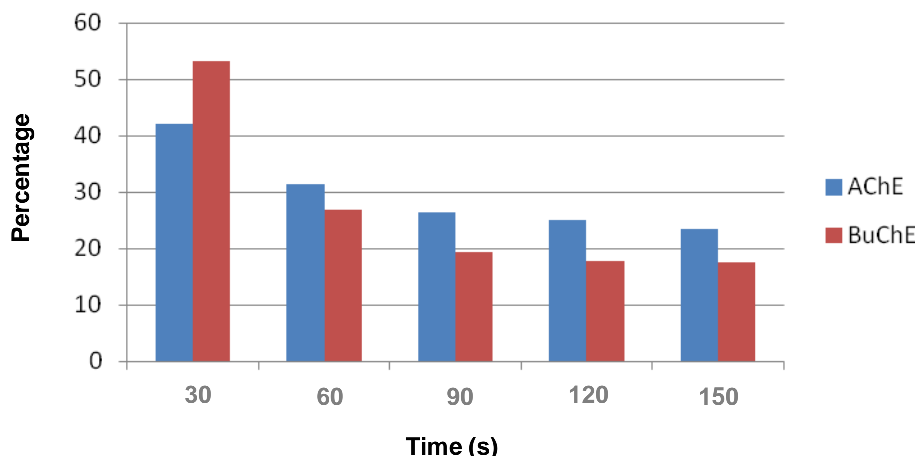

Figure 4. Percentage inhibition of subraction D.

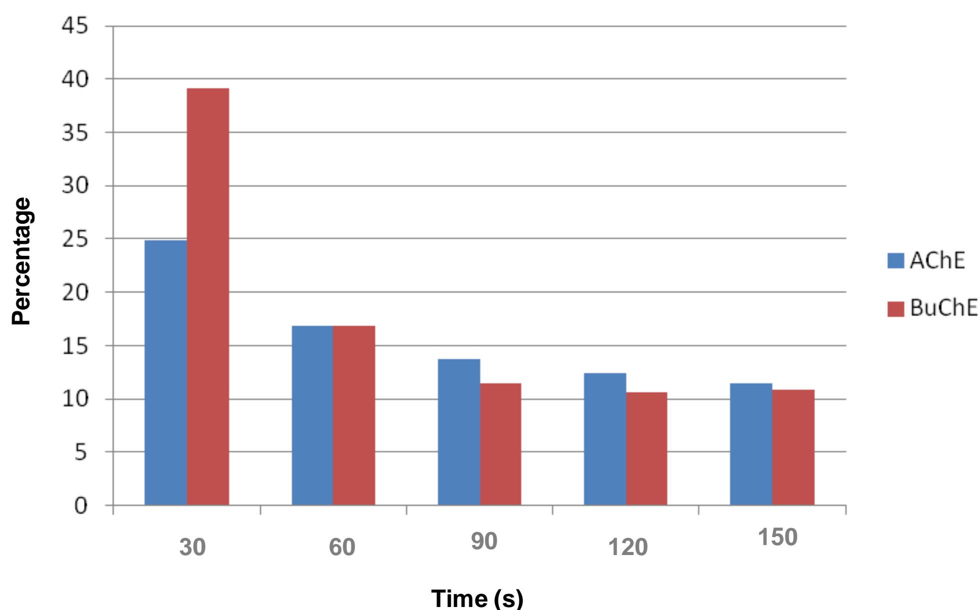

Figure 5. Percentage inhibition of subfraction E.

irrespective of the concentration of the fraction. The results as presented in Figures 1 to 5 showed that subfraction A was most active with a percentage inhibition of 65 and 73.99% against AChE and BuChE, respectively. Activity of subfraction A was time dependent for both enzymes. However, inhibition was more prolonged in AChE. BuChE showed better inhibition at 30 s, but activity dropped more rapidly as the time progressed. Subfraction B showed better BuChE inhibitory activity over the period of time for the reaction. Fractions C to E did not show appreciable activities to both enzymes. It can be seen from the aforementioned that activity increased with purification. It also implicated that the putative AChE and BuChE compounds were likely to be moderately polar.

In conclusion, the findings justified the inclusion of *P. pterocarpum* in therapies for memory loss by traditional medical practitioners and also showed that partial purification enhanced activity of extracts. Further work is however ongoing to identify the active components of the plant.

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Full Length Research Paper

Chemical composition and biological activity of essential oils of Cleopatra mandarin (*Citrus reshni*) cultivated in Egypt

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A comparative study of the essential oils isolated from the leaf and fruit peel of Cleopatra mandarin (*Citrus reshni*) was carried out using gas liquid chromatography (GLC) and GLC/mass analysis (MS). Over 140 components were observed in GLC/MS, of which 123 could be identified. 112 compounds were quantitatively analyzed in the leaf oil with linalool as a major component. The total identified components in the fruit peel oil were 69 and limonene was the most prominent. Cleopatra mandarin volatile components showed high anti-inflammatory activity represented by its effect on tumour necrosis factor-α and nitric oxide. They also showed significant anti-microbial activities against most common Gram positive and Gram negative bacteria and some fungi.

Key words: Cleopatra mandarin, essential oils, gas liquid chromatography (GLC), GLC/mass analysis (MS), anti-inflammatory, antimicrobial.

INTRODUCTION

Plants belonging to genus *Citrus* are known for their nutritional value, unique flavour and medicinal properties. The members of this genus are characterized by many biologically active secondary metabolites such as flavonoids (Tripoli et al., 2007), limonoids (Manners, 2007), coumarins and furanocoumarins, sterols (Ladaniya, 2008), volatile oils (Espina et al., 2010; Tranchida et al., 2011), organic acids and alkaloids (He et al., 2010). Many *Citrus* species are recognized for their medicinal, physiological and pharmacological activities including antimicrobial (Espina et al*.,* 2010; Singh et al*.,* 2010), antioxidant (Barros et al., 2012; Goulas and Manganaris, 2011), anticancer (Benavente-Garcia and Castillo, 2008; Manthey and Guthrie, 2002), anti-inflammatory (Menichini et al. 2011) and hypoglycaemic (Aruoma et al., 2012) activities.

The original source of Cleopatra mandarin was in India and introduced to Florida from Jamaica in the 19th Century. The fruit is orange-red in colour with thin and rough peel and acidic flavour. A survey of the literature revealed very few reports on the identification of secondary metabolites in Cleopatra mandarin. Lota et al. (2001) reported the presence of 29 and 44 volatile components in the fruit peel and leaf oil of Cleopatra mandarin growing in France, respectively. The hydrodistilled oil of the fruit peel gave limonene as the major component (93.6%) followed by myrcene (1.7%), sabinene (1.1%) and linalool (1%). Sabinene (49.7%) was the prominent constituent in the leaf oil followed by linalool (13%) and (*E*) β-ocimeme (6.9%) (Lota et al., 2001). Few reports discussed the biological importance of Cleopatra mandarin constituents. The presence of various polymethoxy flavones contributes to the antifungal properties of the plant (Uckoo et al., 2011), in addition, the plant is used for treatment of capillary fragility, haemorrhages and hypertension due to the presence of high percentage

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of hesperidin (Cano and Bermejo, 2011).

There has been no detailed research on the volatile components of Cleopatra mandarin grown in Egypt. Therefore, and in continuing to the investigation of the chemical and biological activities of *Citrus* spp. cultivated in Egypt (El-Readi et al., 2010; Hamdan et al*.,* 2010a, b; Hamdan et al*.,* 2011), the chemical profile of the fruit peel and leaf oils of Cleopatra mandarin were analyzed by GLC and GLC/MS and their anti-inflammatory and antimicrobial activities were also assessed in this study.

MATERIALS AND METHODS

Plant

The fresh ripe fruits and leaves of Cleopatra mandarin (*Citrus reshni* Hort. ex Tan.), family Rutaceae were collected on the Research Station of the Faculty of Agriculture (Benha University, Egypt) in March, 2011. The identity of the plants was confirmed by Dr. B. M. Houlyel, Prof. of Pomology, Faculty of Agriculture, Benha University. Voucher specimens were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Egypt.

Isolation of the oil

The fresh leaves and ripe fruit peel (100 g each) of Cleopatra mandarin were separately subjected to hydro-distillation for 6 h using a Clevenger-type apparatus. Both oils were dried over anhydrous sodium sulphate and kept in brown vials in the refrigerator at 4°C until further analyses.

Gas liquid chromatography (GLC) and GLC/mass analysis (MS)

The volatile oil constituents were analysed by high-resolution capillary GLC and GLC-MS. Oil samples (1 µl each, dissolved in 1 ml n-hexane) were injected (1 µl volume) into a gas chromatograph (TRACE GC ULTRA, Thermo Scientific, Milan, Italy) under the following conditions: column, RTX-5MS®fused silica capillary equivalent to DB-5 (30 m × 0.32 mm i.d and 0.25 µm film thickness); He as the carrier gas (2 ml/min); flame ionization detector (FID), temperature (300°C), injection temperature (250°C); oven temperature program: initial temperature at 45°C, 2 min isothermal, 300°C, 4°C/1 min, and then 20 min isothermal; split ratio, 1:15. Retention indices (RI) were calculated with respect to a set of co-injected homologous series of saturated hydrocarbon standards (C10 to C28). Components were quantified as area percentage of total volatiles from GC analyses as shown in Table 1. GLC-MS data were recorded on a Clarus 600 gas chromatograph (Connecticut, USA) equipped with an identical column used for separation and quantification. The capillary column was directly coupled to a quadruple mass spectrometer Clarus 600T. The ionization energy for the mass spectrometer was 70 eV. Split ratio was 1:30; other conditions were identical to those mentioned for GLC.

Identification of components

Compounds were identified by comparing their spectral data and RI with Wiley Registry of Mass Spectral Data 8th edition, NIST Mass Spectral Library (December 2005), and literature data (Adams, 2007; Hamdan et al., 2010a). Where possible, retention times and mass spectra were also compared with those of authentic pure samples. Most of the non-identified components are present as traces with relative abundances of less than 0.01%. The identified constituents are listed in the order of their elution in Table 1.

Estimation of tumor necrosis factor-α (TNF-α)

TNF-α was induced in Raw murine macrophage (RAW 264.7) cells using lypo-polysacchride (LPS). The macrophage cells were treated with LPS alone and with the oil samples (100 µg/ml) for 48 h. The level of TNF-α was quantified in the serum produced from the macrophage cells by ELISA. The assay uses the quantitative sandwich immunoassay technique that uses immobilized monoclonal antibody and biotin-linked polyclonal antibody, both of which are specific against mice TNF-α. Commercially available matched paired antibodies were used (R&D Systems Inc. Minneapolis, MN). 4 µg/ml of anti-TNF-α monoclonal antibody and biotin-labelled anti-TNF-α polyclonal antibody (200 ng/ml) were used. The first (capture) antibody was coated onto 96-well flat bottom microtiter plate (Griener Labortechnik, Kremsmunster, Austria) in phosphate-buffered saline (PBS; Sigma Chemical Company, St. Louis, MO, USA), 50 µl/well and incubated 1 h at 37°C, then overnight at 4°C in humidified chamber. Plates were washed three times with washing buffer and blocked with 200 µl/well blocking buffer and incubated at 37°C for 1.5 h. Triplicate assays on 50 µl aliquots of serum samples were quantified by reference to recombinant human standards (R&D Systems, Inc. USA) added to each plate and incubated for 1 h at 37°C. At the end of the incubation period, the plates were washed three times with washing buffer and diluted second biotin labelled antibody was added for 1 h incubation at 37°C. After washing away any unbound substances, the peroxidase-conjugated streptavidin (Jackson Immunsearch Lab, USA) diluted 1:1000 was added to as 50 µl/well, then the plates were incubated for 1 h at 37°C. After an intensive washing, the enzyme reaction was carried out by adding a 50 µl/well of substrate solution. Color development was stopped by addition of 50 µl/well of stopping buffer (1 M HCl) (Surechern Products, Needham Marker, Suffolk, England). The intensity of the developed color was measured by reading optical absorbance at 450 nm using a microplate reader (FLUOstar OPTIMA, BMG LABTECH GmbH, Offenburg, Germany). Enzyme-linked immune sorbent assay (ELISA) reader-controlling software (Softmax) readily processes the digital data of raw absorbance value into a standard curve from which TNF-α concentration of unknown samples can be derived directly.

Estimation of nitric oxide (NO)

Assay of nitrite accumulation (as an indicator of NO production) in the supernatants of cultured RAW 264.7 based on the Griess reaction was done according to Green et al. (1982). The cells were treated with the samples (100 µg/ml) for 48 h and compared with control. The concentration of nitrate was measured by reading the absorbance at 540 nm. The NO level of each of the tested cells was expressed using the following equation: NO level of the tested cells ×100/NO level of the control.

Antimicrobial activity

Cup-plate method (Wood and Washington, 1995) was used to detect the preliminary antimicrobial activity. The test microorganisms: *Staphylococcus aureus* ATCC 6538 (Gram positive bacteria), *Pseudomonas aeruginosa* ATCC 9027*, Klebsiella pneumoniae* ATCC 27736 and *Escherichia coli* ATCC

Table 1. Volatile oil components identified in the fruit peel and leaf oils of Cleopatra mandarin.

Component	RI	Composition (%)	
		Fruit peel	Leaf
α-Thujene*	943	0.09	0.87
α-Pinene•*	947	1.11	2.03
α-Fenchene	957	0.03	0.06
Camphene•*	962	tr.	tr.
Sabinene•*	977	0.98	22.27
β-Pienene•*	999	-	0.03
Myrcene•*	994	3.45	2.91
α-Phellandrene•*	1002	-	0.27
δ-2-Carene	1005	0.74	0.05
α-Terpinene*	1013	0.01	1.41
ρ-Cymene	1022	-	0.52
Limonene•*	1026	79.46	3.78
(Z)-*β*-Ocimene•*	1037	-	0.37
(E)-*β*-Ocimene•*	1049	0.45	5.39
γ-Terpinene•*	1057	-	3.43
cis-Sabinene hydrate	1064	-	0.70
n-Octanol•*	1076	3.33	tr.
ρ-Mentha-2,4(8)-diene	1086	0.37	1.17
Linalool•*	1092	3.26	23.91
trans-Sabinene hydrate•*	1095	-	0.13
trans-ρ-Mentha-2,8-dien-1-ol	1116	0.06	0.08
cis-*ρ*-Menth-2-en-1-ol*	1121	-	0.27
allo-Ocimene	1129	0.01	0.24
cis-*ρ* -Menth-2,8-dien-1-ol	1132	0.02	0.07
trans-*ρ*-Menth-2-en-1-ol	1138	-	0.19
neo-*allo*-Ocimene	1140	-	0.05
cis-*β*-Terpineol	1145	0.02	tr.
trans-Verbenol	1148	-	0.02
Citronellal	1154	0.02	tr.
Karahanaenone	1156	-	0.01
iso-Isopulegol	1160	-	0.01
neoiso-Isopulegol	1165	-	0.01
Terpinen-4-ol•*	1178	0.52	6.32
trans-*ρ*-Mentha-1(7),8-diene-2-ol	1184	-	0.04
α-Terpineol•*	1190	0.84	1.34
Dihydro carveol	1194	0.05	0.09
n-Dodecane	1199	-	0.02
n-Decanal•*	1206	1.01	-
trans-Piperitol	1207	-	0.21
iso-Dihydro carveol	1210	-	0.01
Octanol acetate	1214	0.05	tr.
trans-Carveol	1219	-	0.01
cis-Carveol	1223	-	0.05
Nerol*	1229	0.3	0.09
Thymol, methyl ether	1235	-	0.04
Cumin aldehyde	1238	-	0.05
Geraniol*	1242	0.07	tr.
Piperitone	1253	-	0.01
Methyl citronellate	1258	-	0.06

Table 1. Contd.

Geranial	1273	0.29	0.04
n-Decanol	1275	0.5	-
α-Terpinen-7-al	1283	-	0.03
Limonen-10-ol	1289	0.06	0.04
ρ-Cymen-7-ol	1293	-	0.01
ρ -Mentha-1-en-9-ol	1297	0.03	-
Terpinen-4-ol acetate	1300	0.11	-
n-Tridecane	1301	-	0.01
cis-Piperitol acetate	1326	-	0.02
δ-Elemene	1336	0.05	1.67
α-Cubebene	1347	-	0.04
α-Terpinyl acetate•	1351	-	tr.
Eugenol	1359	-	tr.
Longicyclene	1368	-	0.03
α-Ylangene	1372	0.03	0.05
β-Panasinsene	1382	tr.	0.12
(E)-*β*-Damascenone	1385	0.02	-
β-Elemene*	1390	-	1.73
n-Tetradecane	1398	0.01	0.02
β-Longipinene	1402	-	0.16
Dodecanal	1408	0.14	-
β-Funebrene	1414	0.03	-
(E)-Caryophyllene•*	1416	-	3.65
β-Cedrene	1424	0.02	0.16
γ-Elemene	1431	0.29	-
β-Copaene	1433	-	3.67
α-Guaiene	1437	0.01	0.10
Aromadendrene	1441	tr.	0.07
α-Humulene•*	1451	0.05	0.78
cis-Muurola-4(14),5-diene	1460	0.02	0.05
γ-Gurjunene	1475	-	0.23
γ-Muurolene	1479	0.24	1.01
γ-Himachalene	1483	tr.	-
β-Selinene	1484	-	0.07
trans-Muurola-4(14),5-diene	1489	tr.	-
cis-*β*-Guaiene	1490	-	0.06
γ-Amorphene	1494	0.01	-
α-Alaskene	1495	-	0.57
α-Muurolene	1500	tr.	0.05
γ-Patchoulene	1503	-	0.08
(E,E)-*α*-Farnesene*	1504	0.04	-
δ-Amorphene	1507	-	0.17
γ-Cadinene	1513	tr.	0.06
δ-Cadinene*	1523	0.06	0.31
Z-Nerolidol	1527	0.13	0.03
α-Cadinene	1533	-	0.04
trans-Cadina-1(2),4-diene	1537	0.01	-
cis-Sesquisabinene hydrate	1538	-	0.35
α-Calacorene	1542	-	0.03
Elemol*	1549	0.04	0.11
Germacrene B	1555	0.14	1.77
E-Nerolidol*	1564	-	0.12

Table 1. Contd.

Spathulenol*	1575	-	0.07
Caryophellene oxide*	1579	-	0.09
Carotol	1590	tr.	0.06
n-Hexadecane	1597	tr.	0.04
β-Atlantol	1604	-	0.04
Z-Bisabolol-11-ol	1615	0.01	0.01
10-*epi*-*γ*-Eudesmol	1623	-	0.03
γ-Eudesmol	1628	0.02	0.37
epi-*α*-Muurolol	1642	tr.	0.17
β-Eudesmol	1648	0.02	0.19
α-Eudesmol	1651	0.03	0.11
α-Cadinol*	1653	tr.	0.12
Khusinol	1679	-	0.02
n-Heptadecane	1700	tr.	0.01
Cedroxyde	1713	-	0.02
Mint sulfide	1733	-	0.01
Z-Lanceol	1758	-	tr.
14-hydroxy-*α*-Muurolene	1766	-	0.02
Guaiazulene	1772	-	0.02
α-Eudesmol acetate	1793	tr.	tr.
n-Octadecane	1797	tr.	0.02
Nootkatone	1806	tr.	tr.
Monoterpene hydrocarbons	-	87.04	54.31
Oxygen containing monoterpenes	-	5.97	13.48
Sesquiterpene hydrocarbons	-	1.22	24.15
Oxygen containing Sesquiterpenes	-	0.41	4.27
Others	-	5.04	0.08
Total	-	99.68	96.29

In elution order from RTX-5MS® column. RI = identification based on retention index relative to standard *n*-alkanes. tr. = trace (<0.01 %). - = not detected. *Previously reported in the leaf oil of Cleopatra mandarin (Lota et al., 2001). •Previously reported in the peel oil of Cleopatra mandarin (Lota et al., 2001).

10536 (Gram negative bacteria), *Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 10231(fungi) are standard strains obtained from the Department of Microbiology, Faculty of Pharmacy, Zagazig University, Egypt. The nutrient agar or Sabouraud's agar were seeded by about 10^6 microbial cells. 100 µl of fruit peel and leaf oils were separately dissolved in 500 µl dimethyl formamide (DMF). Each cup was filled by about 100 µl from each solution. Amoxicillin (500 µg/ml) and amphotericin B (500 µg/ml) were used as standard antibacterial and antifungal, respectively. The plates were incubated overnight at 37°C for bacteria and 30°C for fungi. Zones of inhibition were measured (mm).

Statistical analysis

The Student's unpaired *t-test* was used to detect the statistical significance, where a P value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Essential oils obtained from plants have many potential applications, including their use as food additives, preservatives, in perfumes and in pharmaceuticals. These applications are usually due to the medicinal and pharmacological activities of these oils such as antimicrobial, antioxidant and anti-inflammatory activities. Volatile components of the Egyptian variety of Cleopatra mandarin were separated and identified using GLC and GLC/MS and their relative abundance is listed according to their retention indices in Table 1. The oil yields from the fruit peel and leaf were 2 and 0.8% (v/w), respectively. A total of 123 compounds were identified in the fruit peel and leaf oils. The composition of the fruit peel and leaf oils is very different. From the compounds

detected, 67 and 110 components have been identified in the respective oils, representing 99.86 and 96.29% of the oils. In the peel, limonene being the most abundant component (79.46%), this is typical for the orange oils. Only 19 of the identified components were previously reported in the literature and 48 compounds are now reported for the first time as volatile constituents of Cleopatra mandarin. On the other hand, we could not find any traces of *β*-phellandrene, terpinolene, octanal, nonanal, *cis*-limonene-1,2-oxide, octyl acetate, α-copaene, trans-α-bergamotene, neral, germacrene-D and bicyclogermacrene as described by Lota et al. (2001). The main constituents of the leaf oil were linalool (23.91%), sabinene (22.27%), terpinen-4-ol (6.23%) and E-(*β*)-ocimene (5.39%). It is worth noting that 81 compounds are now reported for the first time as volatile constituents of Cleopatra mandarin leaf oil. The compounds 3-carene, *β*-phellandrene, terpinolene, ρ-cymene, bicyclogermecrene, 6-methylhept-5-ene-2-one, nonanal, cis- and *trans*-limonene-1,2-oxide, (E)-*β*-farnesene and manoyl oxide identified previously in the leaf oil of France species (Lota et al., 2001), were not detected in the oil of leaf specimen analyzed in the work. It is evident from the oil yield that seasonal variation, nutrition and temperature have an influence of oil production and its composition.

Anti-inflammatory activity

The anti-inflammatory activity of Cleopatra mandarin oils was determined relative to its ability to inhibit both TNF-α and NO production. TNF-α is an inflammatory mediator which is associated with the development many inflammatory diseases such as rheumatoid, psoriasis and arthritis. Anti-TNF-α is a new approach which is now used in the treatment of many of the former inflammatory diseases (Palladino et al., 2003). NO is another inflammatory regulator which is produced from the amino acid L-arginine through the effect of the nitric oxide synthase. NO is generated through stimulation of many types of cells, especially macrophages, by stimulants such as LPS. If NO is produced in high concentrations out of control, damage of cells occurs due to cell injury. Measuring NO production is a method for assessing the anti-inflammatory effects of essential oils (Kiemer et al., 2002). Many classes of natural compounds have been found to act as anti-TNF-α and NO inhibitor agents. These naturally occurring drugs has the advantage of being safer and sometimes more cost-effective than the chemically-synthesized inhibitors. *Citrus* essential oils are considered to have an anti-inflammatory activity through inhibition of NO production in target area (Yang et al., 2009).

Oils isolated from Cleopatra mandarin reduced the levels of TNF-α and NO in Raw murine macrophage cell culture (RAW 264.7) induced by LPS. The results indicated that the fruit peel oil at concentration of 100 μg/ml, possessed a very high significant inhibitory activity for LPS-stimulated TNF-α and NO levels to the extent nearly of the control level ($P<0.001$). The leaf oil also showed high significant inhibitory activity for LPS-stimulated TNF-α and NO levels ($P<0.01$) as shown in Figure 1. The peel oil showed the higher inhibition activity than leaf oil, respectively as an inhibitor for TNF-α; however, both oils showed nearly the same activity as NO inhibitors. This inhibition activity can be attributed to the presence and the percentage of limonene in the oil. Limonene is reported to suppress the production of TNF-α and NO, thus becoming a potent anti-inflammatory agent especially in skin inflammatory condition (Yoon et al., 2010). Both TNF-α and NO, results and the good yield of oil by hydro-distillation suggests the ability of using Cleopatra mandarin fruit peel oil in skin preparation as an anti-inflammatory potent drug.

Antimicrobial activity

Bactericidal and fungicidal activities are known properties for volatile oils, particularly those of *Citrus* spp. Many studies confirmed the antimicrobial activity of fruit peel oil of many *Citrus* spp. including Cleopatra mandarin (Caccioni et al., 1998; Espina et al., 2010; Fisher and Phillips, 2008). This study investigates the antimicrobial activity of the hydro-distilled essential oil of the fruit peel and the leaf of the Egyptian Cleopatra mandarin. The results revealed that all the tested essential oils had moderate antibacterial effect against all tested Gram positive and Gram negative bacteria as shown in Table 2. The activity ranged from 50 to 66% activity of amoxicillin as a standard broad spectrum antibiotic. The antimicrobial activity was nearly the same on Gram positive and Gram negative bacteria; however, the oils showed good activity against *P. aeruginosa* ATCC 9027 which is known for causing infection in lungs and urinary tract. The examined oils showed relatively strong antifungal activities especially on *A. niger* which reached to 82.6% activity of amphotericin B as an antifungal standard (Table 2). The difference between leaf and fruit peel oils in antimicrobial activities on both the bacteria and the fungi cannot be distinguished. These findings encourage the use of these oils as antimicrobial agents topically or internally which can be considered as a reuse and recycling of a waste product from *Citrus* spp. industries.

Conclusion

Mandarins are one of the fruits which are highly consumed by human due to its nutritional and medicinal values. However, the cultivation and consumption of these fruits generate wastes by-products such as leaves and peel which could bring environmental problems if not properly dealt with. This study was carried out as a step towards reusing and recycling these by-products and

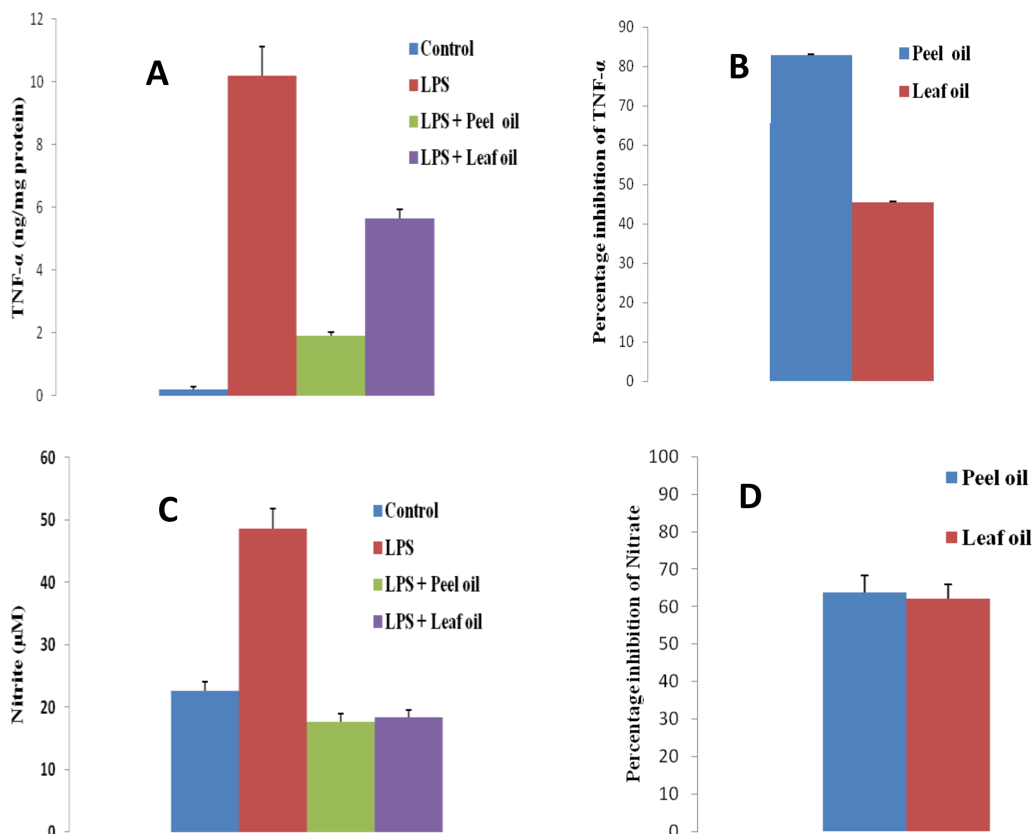

Figure 1. The anti-inflammatory activity of hydro-distilled essential oils of the fruit peel and leaf of Cleopatra mandarin. A: The level of tumour necrosis factor-α (TNF-α) protein in RAW 264.7 cells after treatment with the samples (100 µg/ml) for 48 h compared with lypo-polysacchride (LPS) treated cells as measured by ELISA assay. B: Percentage inhibition of TNF-α by leaf and peel oils. C: The level of nitric oxide (NO) in RAW 264.7 cells supernatant after the treatment with the samples (100 µg/ml) for 48 h compared with LPS treated cells as measured by Griess reaction according to Green et al. (1982). D: Percentage inhibition of NO by the leaf and peel oils. The data are presented as absorbance (mean ±SE) of three replicates (*n*=3).

Table 2. The antimicrobial activity of the fruit peel and leaf oils of Cleopatra mandarin.

Tested material	Activity (%)					
	***Staphylococcus aureus* ATCC6538**	***Escherichia coli* ATCC10536**	***Klebsiella pneumoniae* ATCC27736**	***Pseudomonas aeruginosa* ATCC9027**	***Candida albicans* ATCC10231**	***Aspergillus niger* ATCC16404**
Amoxicillin	100.00	100.00	100.00	100.00	0.00	0.00
Amphotericin B	0.00	0.00	0.00	0.00	100.00	100.00
Leaf oil	58.82	52.86	60.20	62.50	60.71	82.61
Fruit peel oil	44.12	55.56	66.00	64.56	53.57	65.22

Oil samples (100 µl) dissolved in DMF (500 µl) and each cup was filled with 100 µl from each extract. The data is represented as an activity percentage of the standard antibacterial amoxicillin (500 µg/ml) or antifungal amphotericin B (500 µg/ml). The data is the mean of three replicates (*n*=3).

focuses on the identification of new flavors that could have been used in perfumery, food industries and medicinal application.

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Full Length Research Paper

Biochemical effects of leaf extracts of *Gongronema latifolium* and selenium supplementation in alloxan induced diabetic rats

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This study evaluated the effect of selenium and extracts of *Gongronema latifolium* on some biochemical parameters in alloxan-induced diabetic rats. Forty male albino rats equally divided into eight experimental groups were used. Two groups served as normal and diabetic control and received placebo treatment. Four diabetic test groups were treated with *G. latifolium* extract, glibenclamide, combination of *G. latifolium* extract and selenium, and selenium, respectively. Two non-diabetic test groups were treated with *G. latifolium* extracts and selenium, respectively. Treatment lasted for 28 days after which the rats were sacrificed and blood collected for biochemical evaluation. Variations in animal weights were also measured within the period of study. The results showed that treatment with combination of *G. latifolium* extract and selenium significantly reduced ($P < 0.05$) the weight, aspartate transaminase activity and glucose levels and increased superoxide dismutase activity, glutathione-S-transferase activity, albumin and protein levels. Treatment with selenium alone significantly increased catalase activity. There were no significant differences in alanine aminotransferase, alkaline phosphatase and lipid peroxidation.

Key words: Diabetes mellitus, *Gongronema latifolium*, selenium, alloxan.

INTRODUCTION

Diabetes mellitus is a non-communicable metabolic disorder characterized by hyperglycaemia due to overproduction and underutilization of glucose (Ugochukwu et al., 2003; Das and Elbein, 2006; Dharmeshkumar et al., 2008; Srinivasan et al., 2008). Diabetes tends to damage cell membranes which results in elevated production of reactive oxygen species (ROS). The generation of ROS appears to play a critical role in the pathogenesis of diabetes mellitus (Harnett et al., 2000). Hyperglycemia associated with diabetes also increases the production of ROS and affects antioxidant enzymes and reactions (Uchimura et al., 1999; Kowluru et al., 2000; Haskins et al., 2004).

Plants are sources of potential therapeutic agents against various diseasess due to their biodiversity and presence of a wide array of bioactive phytochemicals and secondary metabolites (Farombi, 2003). The use of medicinal plants in management of diseases is as old as mankind and is still an important alternative therapy widely employed in developing countries. Several

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investigations into the chemical and biological activities of plants have yielded compounds with properties useful for the development of modern synthetic drugs for management of several diseases including diabetes (Roja and Rao, 2000; Jung et al., 2006; Malviya et al., 2010; Rao et al., 2010). The anti-diabetic properties of several plants including cinnamon used in treatment and management of diabetes are generally due to various phytochemicals such as polyphenols, catechins, saponins and flavonoids (Bnouham et al., 2006; Khan et al., 2003). Studies have also revealed enhanced efficacy and potency of combination of various anti-diabetic agents in treatment of diabetes (Ebong et al., 2011).

Tropical plants elaborate diverse phytochemicals that are medicinally useful especially in the management of diabetes (Iweala and Okeke, 2005; Pandhare et al., 2012). Amongst them is *Gongronema latifolium* whose individual and synergistic anti-diabetic effects have been reported (Atangwho et al., 2010). *G. latifolium* is a perennial edible shrub of the family, Asclepiadaceae widely employed in Nigeria for various medicinal and nutritional purposes (Ugochukwu et al., 2003; Morebise et al., 2002). Scientific studies have established the hypoglycaemic, cardio-protective, hypolipidaemic, anti-inflammatory and antioxidative effects of aqueous and ethanolic extracts of *G. latifolium* leaf (Edet et al., 2009; Ugochukwu et al., 2003; Ogundipe et al., 2003; Morebise et al., 2002). Some bioactive phytochemicals found in *G. latifolium* which may contribute to its anti-diabetic property include β-sistosterol, lupenyl esters, pregnane ester, glycosides, essential oils and saponins (Ekundayo, 1980; Morebise et al., 2002).

Selenium is a naturally occurring trace mineral required to maintain good health (Marcason, 2008; Rayman, 2008). Selenium is a key component of a number of selenoproteins involved in essential enzymatic functions such as redox homeostasis, thyroid hormone metabolism, immunity and reproduction (Burk, 1998; Diplock, 1994). The redox homeostatic function helps to maintain membrane integrity, protect prostacyclin production and reduce the likelihood of propagation of further oxidative damage to biomolecules which is usually associated with increased risk of diseases (Neve, 1996).

Alloxan, an oxygenated pyrimidine derivative is a toxic glucose analogue, which selectively destroys insulin-producing beta cells in the pancreas when administered to rodents and many other animal species (Lenzen, 2008). This causes an insulin-dependent diabetes mellitus with characteristics similar to type 1 diabetes in humans. Hence, alloxan-induced diabetes in rats is a good experimental model to study diabetes (Szkudelski, 2001).

The established individual roles of *G. latifolium* and selenium in treatment and management of diabetes informed the objective of this preliminary study which was to evaluate the effect of combined administration of both on some biochemical indices of alloxan-induced diabetic rats.

MATERIALS AND METHODS

Collection of plant sample and preparation of extracts

Leaves of *G. latifolium* were bought from a local market in Ota, Ogun state, Nigeria and identified by a qualified plant taxonomist. The leaves were picked and left to dry at tropical room temperature. The dried leaves were picked and ground into a coarse powder using a hammermill. Five hundred grams (500 g) of *G. latifolium* was soaked in 2.5 L of 80% ethanol for 48 h. The mixture was sequentially filtered with chess cloth and Whatman's paper (No.1). The final filtrates were concentrated to a fifth of their original volume in a rotary evaporator under reduced pressure at 40°C and later evaporated to a dried residue in a water bath. The extraction gave a yield of 38 g of crude extract.

Animal procurement and housing

A total of forty male wistar rats aged between 4 to 8 weeks and weighing between 110 to 160 g were used in this study. The rats were obtained from the animal house of the University of Agriculture, Abeokuta (UNAAB), Ogun state, Nigeria and were allowed to acclimatize for three weeks prior to the commencement of the experiments in the animal laboratory of the department of Biological sciences, Covenant University Ota, Ogun state. They were kept in well ventilated and clean cages at an average room temperature of 30°C and their beddings changed every two days. The rats were allowed free access to tap water and fed a standard rat chow throughout the period of the experiment. All the processes involved in the handling and experiment were carried out according to standard protocols approved by the animal ethics committee of the Department of Biological sciences, Covenant University, Ota.

Experimental diabetes Induction

The rats were subjected to a 12 h fast and diabetes induced by intra-peritoneal injection of 150 mg/kg body weight alloxan hydrate (Sigma-Aldrich, U.S.A) reconstituted in normal saline. Three days later, diabetes was confirmed in the alloxan-treated rats that had fasting blood glucose level (FBGL) of 200 mg/dl and above. FBGL was estimated using Fine test glucometer, with blood obtained from the tail vein of the rats.

Experimental design

A total of forty rats were randomly divided into eight groups of five rats each. The normal (NC) and diabetic control (DC) groups received normal saline. Four groups namely GLE, GLI, GLS and SEL were made up of diabetic rats. GLE group was treated with 200 mg/kg body weight of *G. latifolium* extract, GLI group received glibenclamide (an anti-diabetic drug), GLS group was given *G. latifolium* extract (200 mg/kg body weight) and selenium supplement, and SEL group was given selenium supplement only. Two groups namely GLN and SLN were non-diabetic and treated with *G. latifolium* extract (200 mg/kg body weight) and selenium, respectively. Treatments were daily administered on a 12 hourly

basis by orogastric intubation for 28 days. At the end of 28 days, the rats were fasted overnight, euthanized under diethyl ether and sacrificed. Organs including pancreas, liver, and kidney were excised, cleaned by blotting with filter paper and fixed in 10% formyl saline for histological examination. Whole blood was collected by cardiac puncture with sterile needles, placed into sterile tubes and allowed to clot for about 3 h. Clotted blood was centrifuged at 3,000 rpm for 10 min and the serum obtained was pooled. The animal grouping and treatment schedule are shown in Table 1.

Biochemical determinations

Glucose determination

This test was carried out using a glucose enzymatic-colorimetric test kit (GOD-POD), produced by Cypress diagnostics (Belgium). The test principle is based on the oxidation of glucose by glucose oxidase (GOD) to gluconic acid and hydrogen peroxide. The hydrogen peroxide (H_2O_2) forms a red violet color with a chromogenic oxygen acceptor, phenolaminophenazone in the presence of peroxidase (POD). The colour intensity is proportional to glucose concentration in the sample.

Total protein determination

This test was carried out using a total protein test kit produced by Randox laboratories. The test principle involves formation of a coloured complex between cupric ions in alkaline medium with peptide bonds. The intensity of the colour is proportional to the concentration of protein.

Assay for Liver enzymes

Alanine aminotransferase (ALT), Aspartate transferase (AST), and Alkaline phosphatase (ALP) tests were carried out using ultra violet (UV) kinetic test kits produced by Cypress diagnostics**.** The test is based on photometric determination of rate of nicotinamide adenine dinucleotide (NADH) consumption by pyruvate and oxaloacetate which is directly related to ALT and AST activities, respectively.

Determination of albumin

This test was carried out using an albumin test kit produced by Cypress diagnostics. The measurement of serum albumin is based on its quantitative binding to bromocresol green (BCG). The albumin-BCG-complex absorbs maximally at 578 nm and the absorbance is directly proportional to the concentration of albumin in the sample.

Determination of lipid peroxidation

Lipid peroxidation was determined by the thiobarbituric acid reactive substances (TBARS) method as described by Buege and Aust (1978).

Assay of antioxidant enzymes

Catalase (CAT) activity was determined according to the method described by Sinha (1972). SOD activity was determined according to the method described by Zou et al. (1986). Glutathione-S-transferase (GST) activity was determined according to the method described by Habig et al. (1974).

Statistical analysis

The results are presented as mean ± standard error of mean (SEM) and were analysed for statistical significance by one-way analysis of variance (ANOVA). The values with $p < 0.05$ were considered statistically significant.

RESULTS

Body weight, blood glucose, total protein and albumin concentration

Table 2 shows reductions in body weight in GLE (diabetic rats treated with 200 mg/kg body weight of *G. latifolium* extract), GLS (diabetic rats treated with 200 mg/kg body weight of *G. latifolium* extract and selenium supplement), GLN (Non-diabetic rats and treated with 200 mg/kg body weight of *G. latifolium* extract) and SLN (Non-diabetic rats and treated with selenium) groups. There were also significant reductions in the glucose concentration in GLI, GLS, SEL and SLN against the diabetic control. There were no significant changes in the total protein concentration of the test groups as compared to the diabetic control except GLS. There were significant increases in albumin concentrations of GLE and GLS groups.

Activities of liver enzymes

Table 3 shows that the changes in alanine aminotransferase and alkaline phosphatase activities recorded for the test groups were not significantly different from the controls. However, aspartate aminotransferase in GLE, GLI, GLS and SEL groups were significantly reduced.

Lipid peroxidation level and activities of antioxidant enzymes

The results in Table 4 show that lipid peroxidation levels were not significantly different in the test groups except in GLE, where it was increased. There were significant increases in glutathione-S-tranferase activity in GLE, GLI and SEL as compared to the diabetic control. Superoxide dismutase activity was significantly increased in all the diabetic test groups. The results also showed that catalase activity was significantly increased in the SEL group.

Table 1. Animal grouping and treatment.

Group	Class	Number of rats	Treatment	Dosage
NC	Normal control	5	Normal saline	0.1ml
DC	Diabetic control	5	Normal saline	0.1ml
GLE	Diabetic, *G. latifolium* extract treated	5	*G. latifolium* extract	200 mg/kg body weight
GLI	Diabetic, Glibenclamide treated	5	Glibenclamide	-
GLS	Diabetic, *G. latifolium* extract and selenium treated	5	*G. latifolium* extract and selenium	200 mg/kg body weight and 100 mg/kg body weight
SEL	Diabetic, selenium treated	5	Selenium	100 mg/kg body weight
GLN	Non diabetic, *G. latifolium* extract treated	5	*G. latifolium* extract	200 mg/kg body weight
SLN	Non diabetic, selenium treated	5	Selenium	100 mg/kg body weight

Table 2. Blood glucose and total protein levels in control and test groups.

Groups	Class	Body weight change (g)	Blood glucose (mg/dl)	Total protein (mg/dl)	Albumin (mg/dl)
NC	Normal control	4.00±3.41	66.51±8.79	11.38±0.69	2.06±0.28
DC	Diabetic control	10.00±0.00	176.32±0.00	10.70±0.00	1.79±0.00
GLE	Diabetic, *G. latifolium* extract treated	-8.00±18.76[a]	105.23±10.87[a]	10.59±0.64	2.50±0.07[b]
GLI	Diabetic, glibenclamide treated	40.00±0.00[b]	67.86±0.00[a]	9.25±0.00[a]	2.21±0.00
GLS	Diabetic, *G. latifolium* extract and selenium treated	-54.00±0.00[a]	49.89±0.00[a]	12.67±0.00[b]	2.32±0.00[b]
SEL	Diabetic, selenium treated	0.00±10.00	59.73±10.04[a]	11.93±0.02	1.78±0.05
GLN	Non diabetic, *G. latifolium* extract treated	-10.50±23.75[c]	62.53±5.88[c]	11.32±0.58	3.80±0.23[d]
SLN	Non diabetic, selenium treated	-26±8.51[c]	46.58±8.30[c]	11.60±1.20	1.51±0.13[c]

[a]significant reduction with respect to diabetic control; [b]significant increase with respect to diabetic control; [c]significant reduction with respect to normal control; [d]significant increase with respect to normal control.

DISCUSSION

Diabetes mellitus is a disease condition characterised by alterations in carbohydrate, lipid and protein metabolism (Das et al., 1996). The management of diabetes mellitus is considered a global problem because a successful and effective treatment is yet to be discovered. Most of the modern anti-diabetic drugs, including insulin and oral hypoglycaemic agents only control blood sugar levels as long as they are regularly administered and are associated with a number of undesirable effects (Upadhyay et al., 1996; Cheng and Caughey, 2007). This generates the need for better, convenient and less toxic treatment options. The treatment of diabetes mellitus has been attempted with different indigenous plants and polyherbal formulations (Chandel et al., 2011).

The results from this study revealed significant loss of weight of untreated diabetic rats compared to non- diabetic animals. This is attributed to the

Table 3. Activities of liver enzymes in control and test groups.

Groups	Class	ALT (mg/dl)	AST (mg/dl)	ALP (mg/dl)
NC	Normal control	3.68±0.93	15.93±5.61	2.64±0.40
DC	Diabetic control	7.86±0.00	59.5±0.00	1.65±0.00
GLE	Diabetic, *G. latifolium* extract treated	9.84±3.94	12.03±5.48[a]	2.2±0.55
GLI	Diabetic, Glibenclamide treated	7.86±0.00	11.38±0.00[a]	3.3±0.00
GLS	Diabetic, *G. latifolium* extract and selenium treated	13.13±0.00	18.38±0.00[a]	3.3±0.00
SEL	Diabetic, selenium treated	7.44±4.81	87.5±32.38[b]	4.13±2.48
GLN	Non diabetic, *G. latifolium* extract treated	12.47±4.26	71.97±28.59[d]	3.3±0.95
SLN	Non diabetic, selenium treated	10.79±7.77	7.00±1.75[c]	2.48±0.83

[a]significant reduction with respect to diabetic control; [b]significant increase with respect to diabetic control; [c]significant reduction with respect to normal control; [d]significant increase with respect to normal control.

Table 4. Lipid peroxidation levels and activities of antioxidant enzymes in control and test groups.

Groups	Class	Lipid peroxidation (Mol/L)	CAT (Units/L)	SOD (Units/L)	GST (Units/L)
NC	Normal control	5.31±0.67	155.63±3.52	0.14±0.08	1.76±0.90
DC	Diabetic control	3.27±0.00	159.99±0.00	0.01±0.00	0.07±0.03
GLE	Diabetic, *G. latifolium* extract treated	7.45±0.84[b]	181.15±11.49	0.12±0.06[b]	0.80±0.21[b]
GLI	Diabetic, Glibenclamide treated	3.85±0.00	154.47±0.00[a]	0.11±0.00[b]	0.48±0.00[b]
GLS	Diabetic, *G. latifolium* extract and selenium treated	6.41±0.00	148.73±0.00[a]	0.09±0.04[b]	0.24±0.00[a]
SEL	Diabetic, selenium treated	4.13±0.99	202.52±45.34[b]	0.09±0.04[b]	2.16±0.72[b]
GLN	Non diabetic, *G. latifolium* extract treated	6.17±0.54	163.21±45.34[d]	0.07±0.05	1.62±0.40
SLN	Non diabetic, selenium treated	7.06±0.23	162.92±4.82[d]	0.11±0.02	1.32±0.12

[a]significant reduction with respect to diabetic control; [b]significant increase with respect to diabetic control; [c]significant reduction with respect to normal control; [d]significant increase with respect to normal control.

loss in muscle and adipose tissue resulting from excessive breakdown of tissue protein and fatty acids (Granner, 1996). Consumption of *G. latifolium* is not readily associated with increase in weight (Iweala and Obidoa, 2009). Oral administration of extracts of *G. latifolium* and selenium supplementation and their co administration had a hypoglycaemic effect in the diabetic and non-diabetic rats. The group that was administered the anti diabetic drug, glibenclamide, showed a reduction in blood glucose level due to its insulin- stimulating actions on the beta cells of the pancreas (Srinivasan et al., 2008). The enhanced reduction in blood glucose by combined action of *G. latifolium* extract and selenium supplementation may be attributed to the action of selenium which is an insulin mimetic and possibly the ability of the plant extract to alter the inhibitory activity of alloxan on glucokinase which is the glucose sensor of the beta cells (Stapleton, 2000; Steinbrenner et al., 2011). Generally selenium has been found to potentially promote an overall improvement in islet function (Campbell et al., 2008)

Assay for liver enzymes namely ALT, AST and ALP is important in assessing optimal liver function during diabetes. Increase in the level of liver enzymes in the plasma is an indication of liver dysfunction (Dame, 1981). The increase in ALT and ALP were not statistically different from the control group, indicating possible hepato-protective effect of the plant extracts. The group that was administered *G. latifolium* and selenium supplement showed a significant reduction in AST levels as compared to the diabetic control. AST is not a good indicator of liver dysfunction and this further substantiates the possible hepato protective effects of co-administration of the plant extracts and selenium in diabetes.

Total protein and albumin levels were not statistically different between the test groups and the control groups. This could be attributed to low protein content of *G. latifolium* (Atanghwo et al., 2009). However, the increase in total protein and albumin in the group treated with *G. latifolium* and selenium supplement further supports a possible preservation of liver function.

Lipid peroxidation which is one of the characteristic features of chronic diabetes was not significantly changed possibly due to its stabilization by the antioxidant

components of *G. latifolium* and selenium (Lyons, 1991). Catalase which is known to scavenge and detoxify hydrogen peroxide showed a reduced activity in the diabetic control due to increased oxidative stress associated with diabetes. The increase in the activity of catalase in the rats treated with the *G. latifolium* extract and selenium supplement agrees with the study carried out by Atanghwo et al. (2009). *G. latifolium* contains flavonoids and polyphenols which are antioxidants that could bring about reduction in oxidative stress (Iwueke et al., 2010). The results suggest that selenium plays important roles in quenching reactive oxygen species and reduces the oxidative stress associated with diabetes (Diplock, 1994; Mukherjee et al., 1998).

The treatment with glibenclamide elicited a negligible reduction in catalase activity as compared with the co-administration of the plant extract and selenium. The increased activity of SOD and glutathione-S-transferase in the groups administered plant extracts and selenium further confirms their high antioxidant capacities (Ogundipe et al., 2003). Increased activity in SOD by *G. latifolium* has been reported by other studies (Ugochukwu and Babady, 2002; Iweala and Obidoa, 2009). SOD is the major enzyme involved in scavenging of reactive oxygen species (Mahdi, 2002). The increase in the activity of SOD suggests this process as one of the mechanisms by which *G. latifolium* and selenium produces their synergistic anti-diabetic actions.

The increase in GST can be attributed to phyto-chemicals, especially flavonoids and anthocyanidins in *G. latifolium* which induce detoxification enzymes through up regulation of their genes by interacting with antioxidant response elements (ARES) (Birt et al., 2001; Ren et al., 2003; Ross and Kasum, 2002; Ferguson, 2001). The ability of substances to induce GST is due to possession of electrophilic centers that are able to react with sulfhydryl groups through oxido-reduction or alkylation (Prestera et al., 1993). An increase in GST activity translates to increased capacity to conjugate and excrete toxic intermediates that can cause diseases such as diabetes (Pantuck et al., 1984).

The results obtained from this study indicate that the combined administration of *G. latifolium* and selenium supplementation have superior blood glucose level lowering effects comparable to standard anti-diabetic drugs such as glibenclamide (Ugochukwu et al., 2003). This superior effect is possible due to synergism usually associated with bioactive compounds from medicinal plants and other agents (Tiwari and Rao, 2002; Asuquo et al., 2010). The results indicate the beneficial effect of micronutrients combination in controlling diabetic hyper-glycemia (Aly and Mantawy, 2012). The combined administration also showed promises in effective amelioration of complications associated with diabetes which are linked to oxidative stress, liver dysfunction and lipid peroxidation (Atangwho et al., 2010). This will influence the pattern of use of herbal medicine by diabetic patients (Egede et al., 2002).

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Short Communication

Chemical composition of the methanolic leaf and stem bark extracts of *Senna siamea* Lam.

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The study was conducted to evaluate some chemical constituents of the leaf and stem bark of *Senna siamea*. Standard methods were used in all the analysis. Preliminary phytochemical screening indicated the presence of flavonoids, tannins, polyphenols, anthraquinones, saponins, and glycosides. Quantitative study of some phytoconstituents showed a significant difference of ($p < 0.05$) between the leaf contents and that of the stem bark with the exception of tannins. The results of elemental analysis revealed that the levels of potassium (K), sodium (Na), magnesium (Mg) and manganese (Mn) differed significantly ($p < 0.05$) when leaf contents were compared with that of the stem bark, while nickel (Ni), chromium (Cr), iron (Fe) and copper (Cu) contents were not statistically ($p < 0.05$) different. This result indicated that *S. siamea* has great potentials as it contains active pharmaceutical ingredients.

Key words: *Senna siamea*, phytochemicals, elemental compositions.

INTRODUCTION

Medicinal plants contain physiologically active constituents, which over the years have been exploited in traditional medical practice for the treatment of various ailments (Okigbo and Igwe, 2007). Plant extracts from them have been used for the cure of many disease conditions, and some of their effectiveness have been established (Sofowora, 1993). Researches are currently being conducted on medicinal plants/extracts to isolate and purify the active fractions for preparation of drugs from natural sources (El-mahmood and Amey, 2007) due to their less toxic effects and affordability (Mohammed et al., 2010). *Senna siamea* Lam. (Irwim and Barneby-*Cassia siamea* Lam.) (Fabaceae, Caesalpiniaceae) (El-mahmood and Doughari, 2008), or in Hausa as ''Malga'' (Bala, 2006), was introduced to Africa from tropical Asia. It is widely grown throughout tropical Africa. It belongs to Caesalpiniacea (Von maydell, 1986). *S. siamea* has been reported to be used in the management of constipation, diabetes, insomnia (Tripathi and Gupta, 1991), hypertension, asthma, typhoid fever, and diuresis (Hill, 1992). Leaves and bark of medicinal plants were reported to be used locally as antimalarial medications (Lose et al., 2000). The flowers and young fruits were used as curries (Kiepe, 2001).

This work was undertaken to investigate the phytochemical constituents and elemental compositions of the leaves and stem bark of *S. siamea* against the background of the uses of the plant parts by traditional herbalists.

MATERIALS AND METHODS

Plant collection and extraction

Leaves and stem bark of *S. siamea* were collected in April, 2005 within the premises of Bayero University, Kano. The plant parts were authenticated by botanists in the Department of Biological Sciences, Bayero University, Kano. Leaves and stem bark were

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Table 1. Qualitative phytochemical screening of the leaves and stem bark of *S. siamea.*

Phytochemicals	Leaves	Stem bark
Anthraquinones	+	+
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Polyphenols	+	+
Glycosides	+	+

(+) detected.

Table 2. Quantitative phytochemical constituents of *S. siamea.*

Samples (g/100 g)	Leaves	Stem bark
Alkaloids	10.5±0.71[a]	4.75±0.21[b]
Saponins	8.1±0.35[a]	4.4±0.28[b]
Flavonoids	19.3±0.57[a]	12.4±1.57[b]
Tannins	4.8±0.28[a]	5.9±0.28[b]

All values are means ± SD of four replicates. Values with different superscripts along a row are statistically different ($p < 0.05$).

each separately washed, wiped-dry, sun-dried, cut into small pieces and subsequently reduced to coarse powder. Fifty grams of each leaves and stem bark were separately extracted overnight with methanol, with intermittent vigorous shaking. Each extract was filtered, concentrated with a rotary evaporator and dried over a water bath at 45°C. The residue from each plant part was used for phytochemical screening.

Phytochemical analysis

Methanolic extracts of each plant parts were used for preliminary phytochemical analysis using standard methods; alkaloids, saponins, flavonoids (Sofowora, 1993); anthraquinones (Harbone, 1973) and tannins (Trease and Evans, 1978). For the quantitative determination of some phytochemicals, the method of Bohm and Koupai-Abyazani (1994) was used for flavonids, while alkaloids, saponins and tannins were analysed using that of Wasagu et al. (2005).

Elemental analysis

Elemental composition of each plant part was carried out on the ash sample obtained by the dry-ashing method (Tracey, 1980). The ash sample from each plant part was quantitatively transferred to a 500 cm^3 beaker, using distilled water of 100 cm^3. Concentrated hydrochloric acid of 10 cm^3 was added and the solution boiled for several minutes. After cooling, each solution was then diluted to 500 cm^3 and then filtered. The resulting solution was used to measure the absorbance of elements analysed using atomic absorption spectrophotometer (model sp 2900); sodium and potassium were determined using a flame photometer (model Gallen-kamp FGA-300-C).

RESULTS AND DISCUSSION

Preliminary qualitative phytochemical screening of the leaves and stem bark of *S. siamea* revealed the presence of anthraquinones, alkaloids, tannins, polyphenols, glycosides, saponins and flavonoids in both the leaves and stem bark (Table 1). To further ascertain the preliminary result, a quantitative estimation of some phytochemicals were carried out, and the result showed that the leaves content were significantly ($p < 0.05$) higher than that of stem bark (Table 2). These active phytochemicals are known for their medicinal activity as well as physiological actions; as such they confer the therapeutic potentials of all medicinal plants. Alkaloids, saponins, and tannins have been reported to inhibit bacterial growth and protective to plants against fungal infections (Doughari and Okafor, 2008). Anthraquinones were reported to be used as a laxative (Amadi et al., 2006). Flavonoids were reported to suppress tumour growth and prevent blood clots (Seyfulla and Borisora, 1990). Thus, the medicinal uses reported of *S. siamea* in managing constipation, its antimicrobial and antimalarial uses may be attributed to the presence of these phytochemical constituents. These results are in accordance with the previous report (Smith, 2009). Although, Bukar et al. (2009) reported the absence of flavonoids, saponins and alkaloids in ethanolic and chloroform extracts, it could be as a result of different solvent used.

In addition to phytochemical composition, the elemental profile of medicinal plants is another important factor that determines the medicinal value of these plants. The levels of Mn, Cr, Mg, Na, and K (mg/100 g) of *S. siamea* leaves (Table 3) differ significantly ($p < 0.05$) from that of the stem bark, while Cu, Pb, Ca, Zn and Ni in the leaves content did not differ significantly from that of the stem bark. The need for supplementary diet rich in these minerals cannot be overemphasized for many reasons; they play a vital role for man and other animals as curative and preventive agents in combating diseases, nutritive and catalytic disorders (Abulude et al., 2006). They also help towards the catalytic activity of many enzymes and hormones (Nzikou et al., 2010). Thus, intake of these elements should be such that does not lead to any form of health disorder as some clinical abnormalities were reported due to high level of some minerals. The result of this work agrees with the report of Ingweye (2010).

Conclusion

The result of this study supports the fact that *S. siamea* carries some active biocomponents that have therapeutic potentials, and as well support the local uses of this plant. However, these local medicinal uses are subject to further scientific verification.

Table 3. Elemental composition of the leaves and stem bark of *S. siamea.*

Elements (mg/100 g)	Leaves	Stem bark
Copper	0.49±0.11[a]	0.69±0.10[b]
Iron	6.74±0.17[a]	5.51±0.25[a]
Manganese	0.72±0.24[a]	0.88±0.18[b]
Lead	0.06±0.02[a]	0.11±0.03[b]
Chromium	1.49±0.08[a]	1.05±0.11[b]
Nickel	2.99±0.38[a]	3.36±0.25[a]
Calcium	87.72±1.75[a]	96.49±1.76[b]
Zinc	11.08±9.14[a]	17.99±8.01[a]
Magnesium	126.31±0.61[a]	47.29±0.90[b]
Sodium	350.88±87.72[a]	263.16±87.72[b]
Potassium	257.01±19.90[a]	116.82±4.70[b]

All values are means ± SD of four replicates. Values with different superscripts along a row are statistically different ($p < 0.05$).

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