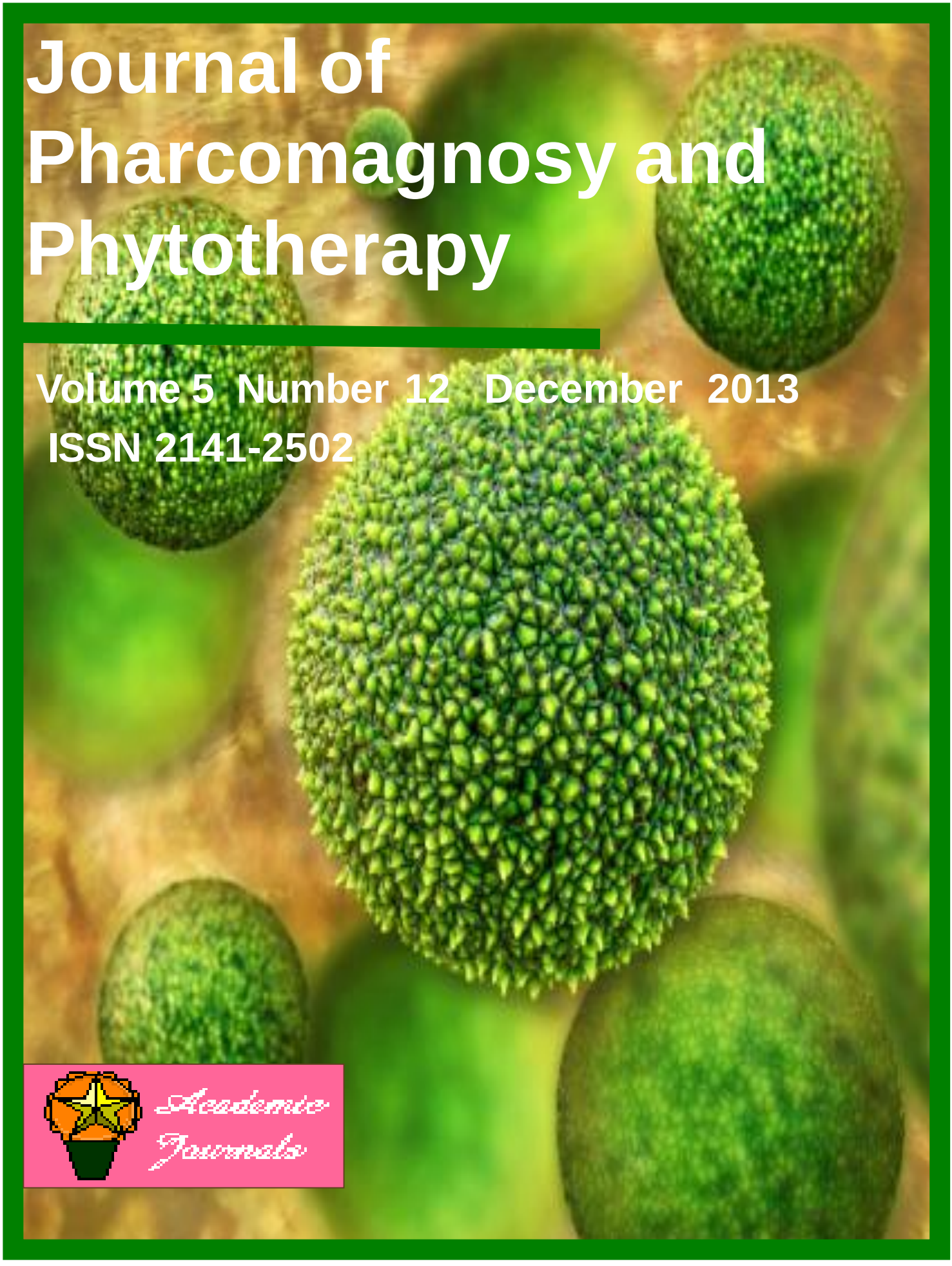

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Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds), Pharmaceutical Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International. pp 181-190.

Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for Striga suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

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Full Length Research Paper

In vitro potential anthelmintic activity of bulbils of *Dioscorea bulbifera* L. on earthworms and liverflukes

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Accepted 15 November, 2013

Ethnobotanical information from Nigeria specifies the usage of *Dioscorea bulbifera* L. (Dioscoreaceae) in treatment of parasitic diseases in human and thus, could be of value in preventing the development of resistance to common synthetic anthelmintics. The present study was designed to evaluate the *in vitro* anthelmintic activity of methanolic extracts of the flesh and peel of the bulbils of *D. bulbifera*, on *Fasciola gigantica* and *Pheritima posthuma* at concentrations ranging from 10 to 100 mg/ml. Albendazole and normal saline were included in the assay as standard reference drug and control, respectively. Thin layer chromatography was used to screen the methanol extracts of the flesh and peel of the bulbils of *D. bulbifera* for important secondary metabolites in comparison with gallic acid and quercetin. The median lethal concentration values of the flesh and peel extracts of *D. bulbifera* were 39.67 and 30.40 mg/ml for earthworm and 61.73 and 41.79 mg/ml for liverfluke, respectively. The peel was more potent at 100 mg/ml, causing paralysis in 5.6 ± 0.51 min and death in 10 ± 0.45 min in earthworm. The findings from this study show that *D. bulbifera* possess *in vitro* anthelmintic compound worthy of further evaluation.

Key words: Albendazole, anthelmintic, *Dioscorea bulbifera*, *Fasciola gigantica*, *Pheritima posthuma.*

INTRODUCTION

The incidence of helminth infections is a global human health concern. Tropical regions of the world, particularly the Sub-Saharan African communities are among the worst hit by the diseases (Hotez et al., 2007). The majority of infections due to helminths causes enormous hazard to health, contributing to the prevalence of under nourishment, anaemia, eosinophilia and pneumonia (Bundy, 1994). Parasitic diseases such as lymphatic filariasis, onchocersiasis and schistosomiasis cause ruthless morbidity affecting principally population in endemic areas (Tagbota and Townson, 2001). The parasitic gastroenteritis is caused by mixed infection with several species of stomach and intestinal worms which result in weakness, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity (Gibbs, 1986).

The use of synthetic anthelmintic drugs is part of important worm control strategy throughout the world. Ideally, an anthelmintic agent should have broad spectrum of action, high percentage cure with a single therapeutic dose, non-toxic to the host and should be cost effective (Ekeanyanwu and Etienjirhevwe, 2012). However, most of the common synthetic anthelmintics drugs available in the market are lacking in these requirement (Mali and Mahta, 2008). A contemporary challenge in the treatment of helminths diseases is the development of resistance by

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the parasites against conventional anthelmintics (Walter and Prichard, 1985; Geert and Dorny, 1995; Cole, 1997; Tagbota and Townson, 2001). Also, the high cost of modern anthelmintics has limited the effective control of these parasites and thus, has led to the screening of many medicinal plants for their anthelmintic activity in search for newer anthelmintic drugs (Akhtar et al., 2000; Abdel-Ghaffar et al., 2011; Klimpel et al., 2011; Tandon et al., 2011).

Herbal drugs have been in use since ancient times for the treatment of parasitic diseases in humans such as lymphatic filariasis, onchocersiasis, schistosomiasis and could be of value in preventing the development of resistance (Hammond et al., 1997). Alkaloids, flavonoids, saponins and tannins have been demonstrated to possess anthelmintic activities (Ekeanyawu and Etienajirhevwe, 2012). In their findings, moderately high amount of tannin was reported in the aqueous, ethanol and methanol extracts of *Monodora myristica* and *Xylopia aethiopica* seeds and was possibly responsible for the significant anthelmintic activity. Chemically, tannins are polyphenolic compounds (Niezen et al., 1995). They also suggested that the presence of steroidal alkaloids oligosaccharides may have suppressed the transfer from the stomach to the small intestine which could diminish the availability of glucose to helminths together with its antioxidant effect which is capable of reducing the nitrate generation.

Alkaloids have been reported to act on the central nervous system of earthworms causing paralysis (Roy, 2010). Similarly, Nayak (2010) on the basis of phyto-chemical analysis and anthelmintic results of the crude extracts of *Hyptis suaveolens* suggested that phenolic content in the extracts produced similar effects as some synthetic phenolic anthelmintics like niclosamide, oxy-clozanide and bithionol which are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1994).

Dioscorea bulbifera, besides being important as an edible yam, is reported to have diuretic activity (Dhawan et al., 1997). Traces of diosgenin have also been detected in this species (Quigley, 1978). *D. bulbifera* is widely used in traditional Indian and Chinese medicine in the treatment of sore throat, gastric cancer and carci-noma of rectum and goiter (Gao et al., 2002). The various extracts of bulbils of the plant have been reported to be antihyperlipidemic (McKoy et al., 2003), antitumour (Gao et al., 2002), antioxidant (Jindal et al., 1969), analgesic and anti-inflammatory (Nguelefack et al., 2011), plasmid curing (Shririam, 2008) and antihyperglycemic (Ahmed et al., 2009). *D. bulbifera* is used in Bangladesh for the treatment of leprosy and tumours (Murray et al., 1984) and by the native people of the Western highlands of Cameroon for the treatment of pig cysticercosis. In Zimbabawe, the plant is used as an infusion applied on cuts and sores, both for humans and animals while in Cameroon and Madagascar, the powdered bulbs are applied to abscesses, boils and wound infections (Cogney, 2002). In India, its bulbils are used to treat piles, dysentery, syphilis, ulcers, pain and inflammation (Gupta and Singh, 1989).

Ethnobotanical information obtained from the field of collection of *D. bulbifera* in Southwest Nigeria revealed that the powdered bulbil soaked in water is effective in reducing high blood pressure and that when it is roasted and eaten by farmers during the scarcity of other yam species, it destroys and expels microbes and parasites through the faeces. In view of the ethnomedicinal application of *D. bulbifera*, the present study was designed to evaluate the *in vitro* anthelmintic activity of methanol extracts of the flesh and peel of the bulbils of the plant.

MATERIALS AND METHODS

Plant collection and authentication

Bulbils of *D. bulbifera* were collected in Ibadan (7.40°N, 3.92°E; tropical wet and dry climate), Southwest, Nigeria in November, 2011. The authentication of the plant was done by Mr. O. A. Osiyemi at the Forest Herbarium Ibadan (FHI) where the voucher specimen FHI 109529 was deposited. Bulbils were separated into flesh and peel, dried under shade and ground into powder with the aid of an electric mill. The powder was stored in air-tight container at 4°C until use.

Plant extraction

Five hundred grams of powdered flesh and peel of the bulbils of *D. bulbifera* were macerated separately in a 2 L flask using redistilled methanol as solvent for a period of 72 h with intermittent stirring with a glass rod and filtered using filter paper (Whatman No. 1, Whatman® Schleicher and Schuell). The combined filtrates were concentrated using Rotavapor (Rotavapor R-210; Buchi Rotavapor) at a temperature of 40°C under reduced pressure. The extracts were stored at 4°C until needed for analysis.

Phytochemical screening

Standard phytochemical tests were carried out on the crude extract of the flesh and the peel of *D. bulbifera* to detect the presence or absence of carbohydrates, cholesterol, alkaloids, sterorids/ triterpenoids, tannins, flavonoids, anthraquinones, cardiac glycosides and saponins (Trease and Evans, 2002; Harborne, ; Sofowora, 2008).

Preliminary thin layer chromatography (TLC) screening

Thin layer chromatography (TLC) was further used to screen the methanol extracts of the peel and flesh of bulbils of *D. bulbifera* for important secondary metabolites using pre-coated TLC plates (Silica gel G 60 F_{254} sheets 20 × 20 cm, 0.5 mm thickness, Merck). The extract and reference compounds (gallic acid and quercetin) were spotted on TLC plates and subsequently developed in suitable

solvent system containing ethyl acetate, methanol, ethanol and water in ratio 81: 11: 4: 8. The plates were dried, visualized in daylight and under ultraviolet (UV) lamp fluorescence at 254 and 365 nm before they were sprayed with 1% anisaldehyde in glacial acetic acid and 5% ferric chloride in 0.5 N HCl (Gage et al., 1951).

Test organisms

Liver flukes (*Fasciola gigantica*, 2.2 to 4.4 cm in length) were obtained from freshly slaughtered cattle in the Bodija abbatoir, in Ibadan metropolis (7.40°N, 3.92°E). Earthworms (*Pheritima posthuma*, 5.5 to 12.5 cm in length) were collected from the water logged areas of Coca-Cola, Sango, Ibadan. Identification and authentication of worms were done by Dr. Soji Abiola of the Department of Veterinary Medicine, University of Ibadan. *P. pasthuma* was used due to its anatomical and physiological resemblance with parasitic gastrointestinal nematodes in human being (Nirmal et al., 2007).

Anthelmintic bioassay

The anthelmintic study of the flesh and peel extracts against the selected worms (*P. posthuma* and *F. gigantica*) was conducted according to the method described by Ajaiyeoba et al. (2001) with slight modifications. Plant extract (10 g) was dissolved in saline water to make stock solution and different concentrations of (100, 70, 50, 20 and 10 mg/ml) were prepared for the anthelmintic assay. Albendazole (10 mg/ml) was included as reference drug, while saline water was included as control. Standard drug and extract solutions were freshly prepared before starting the experiment. For the evaluation of each plant extract, five worms (same type) were placed in a 9 cm Petri dish containing 25 ml solution of methanol crude extracts of plant in the tested concentrations. The plant extract was dispensed into the Petri-dish before introducing the worms. Observations were made for the time taken until paralysis and death of an individual worm. Mean time for paralysis (P in min) was taken when no movement of any sort could be observed, except when the worms were shaken vigorously. Times of death of worms (D in min) were recorded after ascertaining that worms neither moved when shaken rigorously nor when dipped in warm water (50°C). The LC_{50} was determined using a linear regression.

Determination of median lethal concentration (LC_{50})

The worms were divided into twelve groups comprising five worms in each group. Groups 11 and 12 served as positive and negative control and received albendazole and saline water, respectively. Groups 1 to 5 of earthworms and groups 6 to 10 of liverflukes were treated with 10, 20, 50, 70 and 100 mg/ml dose of plant extract. Time of paralysis and death was observed for 24 h post-administration of the extract. From these observations, the median lethal concentration (LC_{50}) of the extract was calculated using Microsoft excel 2007.

Statistical analysis of result

All data were presented as mean ± S.E.M using Microsoft Excel 2007. Statistical analysis was performed using independent Student t-test (Graph Pad Prism version 6.0). Mean time of paralysis and death of worms were considered statistically significant at $P<0.05$.

RESULTS

The flesh and peel extracts of *D. bulbifera* showed significant anthelmintic activity at 100 mg/ml. The time of paralysis (P) and death (D) of the worms are presented in Figures 1 and 2. Anthelmintic activity was higher in the peel extract with LC_{50} values of 30.40 and 41.79 mg/ml and coefficient of determination (R^2) of 0.640 and 0.728 for earthworms and liverfluke, respectively (Figures 3 and 4). Earthworms were more sensitive to the peel extract of *D. bulbifera* as shown in Table 1. The peel extract produced paralysis in 5.6 min and death in 10 min while flesh extract showed paralysis in 8.4 min and death in 13.8 min at 100 mg/ml, when P and D for the reference drug (Albendazole) were 15.2 and 39.6 min, respectively at 10 mg/ml. Similarly, the peel extract exhibited significant anthelmintic properties with liverfluke. Liverflukes were paralyzed after 10.2 min and died after 15.81 min at 100 mg/ml whereas P and D for the reference drug were 28 and 50.6 min, respectively at 10 mg/ml. In this experiment, it took a longer time for the earthworms and liverflukes to die in both the flesh and the peel methanol extracts at 10 mg/ml. The preliminary phytochemical screening revealed the presence of phenolics (tannins, flavonoids), saponins as well as other secondary metabolites.

Thin layer chromatography analysis on pre-coated silica gel plates showed the presence of five to seven spots, representing different compounds in the methanol extracts of peel and flesh of *D. bulbifera* (Table 2). Phenolics and flavonoids were corroborated with the use of TLC which indicated their presence by showing spots on the plates having the same R_f values with reference compounds that is, gallic acid and quercetin. Spraying of the developed TLC plate with 5% ferric chloride in 0.5 N HCl showed a light blue colour and R_f value of 0.86 for both flesh and peel of *D. bulbifera* under UV lamp, 254 nm. This was comparable with the deep blue colour and R_f value of 0.83 observed with gallic acid under the same wavelength. The blue colour with almost similar R_f value indicate the presence of phenols in the extract.

DISCUSSION

Anthelmintic drugs are known to act by causing paralysis of worms or damaging cuticle, leading to partial digestion or to injection by immune mechanism. It also interferes with metabolism of worms since the metabolic requirements of these parasites vary greatly from one species to another (Aisawanya et al., 2010). Albendazole has been shown to affect worms by destroying the cytoskeletal structure of the worm thereby causing paralysis (Nikesh et al., 2011). Also, the predominant effect of piperazine citrate in worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis (Sini et al., 2011).

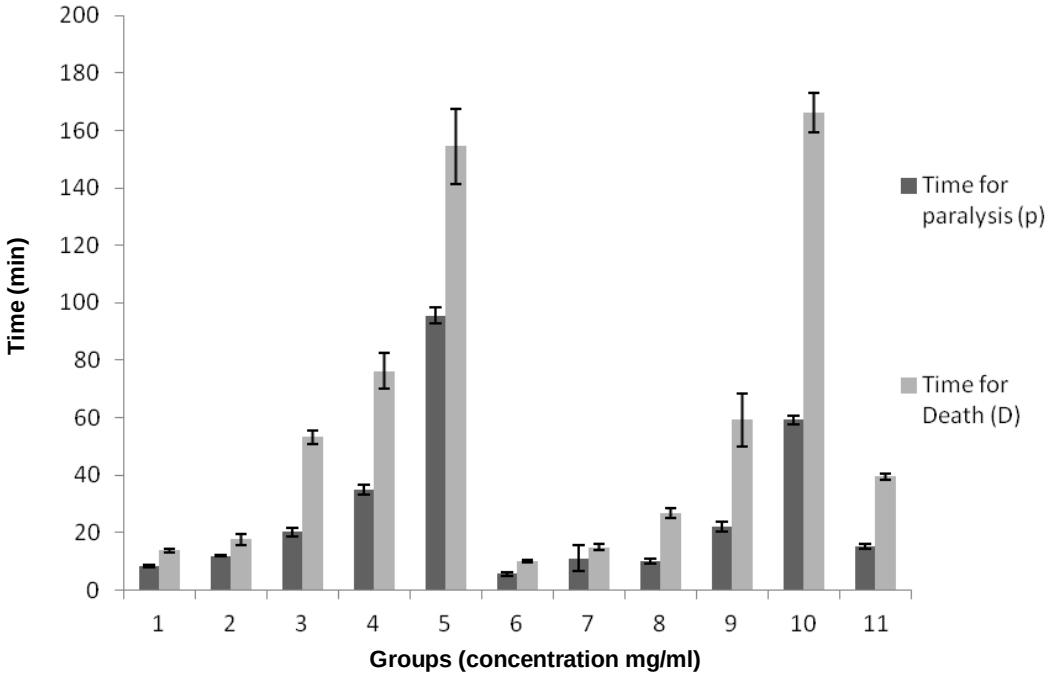

Figure 1. Anthelmintic Activity of Methanol Extracts of flesh and peel of bulbils of *Dioscorea bulbifera* using *Pheritima posthuma*. Groups (1 to 5) 100, 70, 50, 20 and 10 mg/ml of flesh of bulbils of *D. bulbifera*; Groups (6 to 10) 100, 70, 50, 20 and 10 mg/ml of peel of bulbils of *D. bulbifera* and Group (11) 10 mg/ml Albendazole as standard drugs.

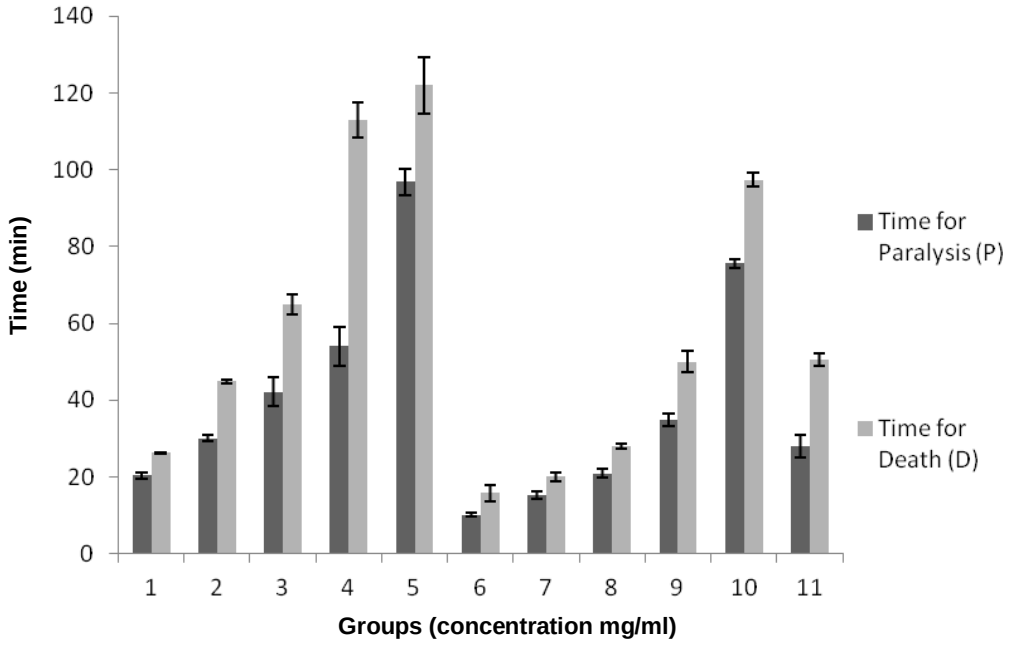

Figure 2. Anthelmintic Activity of methanol extracts of flesh and peel of bulbils of *Dioscorea bulbifera* using *Fasciola gigantica.* Groups (1 to 5) 100, 70, 50, 20 and 10 mg/ml of flesh of bulbils of *D. bulbifera;* Groups (6 to 10) 100, 70, 50, 20 and 10 mg/ml of peel of bulbils of *D. bulbifera* and Group (11) 10 mg/ml Albendazole as standard drugs

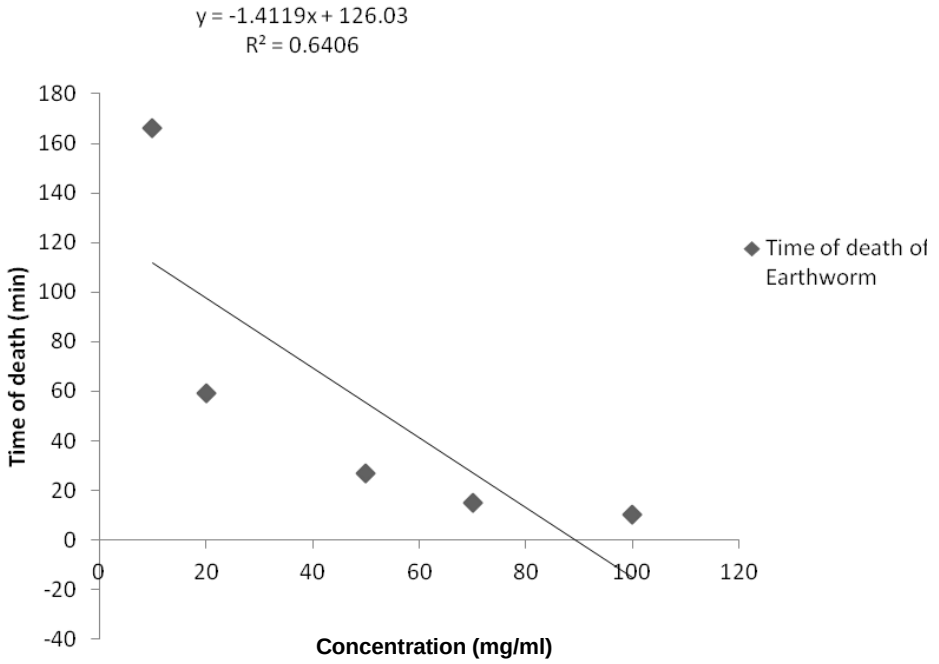

Figure 3. Regression equation of time of death of earthworms against concentration.

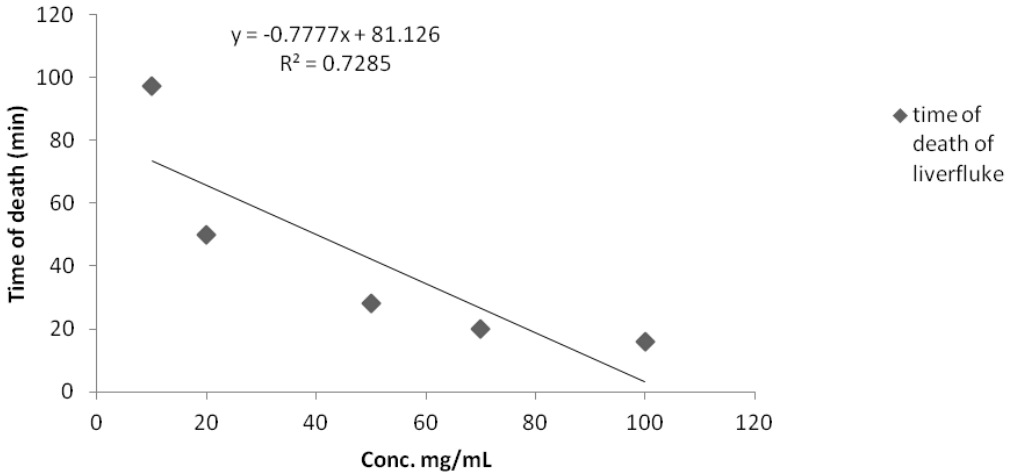

Figure 4. Regression equation of time of death of liverfluke against concentration.

Ekeanyanwu and Etienejirhevwe (2012) reported moderately high amount of tannin (phenolic compounds) in the aqueous, ethanol and methanol extracts of *Monodora myristica* and *Xylopia aethiopica*. They suggested that it could be responsible for the anthelmintic activity observed in their study in that it acts in a similar way to synthetic phenolic anthelmintic like niclosamide, oxyclozanide and bithionol. These compounds are known to interfere with energy generation in helminth parasites by uncoupling parasite specific reductase mediated oxidative phosphorylation reaction. Phenolic compounds were also reported to be responsible for anthelmintic activity in the evaluation of the root of *Raphanus sativus* (Devraj, 2011). Phenolic and tannin compounds show anthelmintic activity by binding to glycoprotein on the cuticle of the parasite and thus lead to death of the worm (Kane, 2009). The extracts in the present study may have demonstrated this property resulting to subsequent death of

Table 1. Anthelmintic activity of methanol extracts of flesh and peel of the bulbils of *Dioscorea bulbifera. Pheritima pasthuma Fasciola gigantica*.

Extract	Concentration (mg/ml)	P	D	P	D
Flesh of *D. bulbifera*	10	95.60±2.87*	154.40±13.16*	96.80±3.38*	122.0±7.37*
	20	35.00±1.70*	76.20±6.20*	54.00±4.94*	113.0±4.64*
	50	20.20±1.43	53.20±2.23*	42.20±3.83	64.80±2.63*
	70	12.00±0.32	17.60±1.75	30.10±0.71	45.00±0.50
	100	8.40±0.40	13.80±0.58	20.40±0.89	26.30±0.12
Peel of *D. bulbifera*	10	59.20±1.35*	166.20±6.89*	75.60±1.04*	97.30±1.82*
	20	22.20±1.74*	59.20±9.06	35.00±1.60*	50.00±2.86
Albendazole	50	10.20±0.86	26.80±1.88	21.00±1.14	28.00±0.71
	70	11.00±4.51	15.00±1.26	15.20±1.04	20.10±1.12
	100	5.60±0.51	10.00±0.45	10.20±0.50	15.81±2.13
	10	15.20±1.02	39.60±1.08	28.00±2.82	50.60±1.72

Time of Paralysis (P) and Death (D) of worms in minutes In the control (Normal saline treated) *P. posthuma* lived 36 h while *F. gigantica* lived 41/2 h. Values are expressed as mean ± SEM (n=5) *Means significantly different at P<0.05 compared with the Albendazole treated group in each column using independent student t- test

Table 2. Thin layer Chromatography of crude extract of the flesh and peel of bulbils of *Dioscorea bulbifera.*

Plant extract	Adsorbent	No. of Spot	R_f 1	R_f2
Flesh	Silica gel	1	0.34	0.64
		2	0.59	0.69
		3	0.71	0.77
		4	0.79	0.86
		5	0.87	0.96
		6	0.91	-
Peel	Silica gel	1	0.31	0.64
		2	0.59	0.69
		3	0.69	0.77
		4	0.81	0.86
		5	0.84	0.89
		6	0.87	0.91
		7	0.91	0.96
Quercetin	Silica gel	1	0.82	0.94
Gallic acid	Silica gel	1	0.69	0.83

R_f 1: retardation factor 1 (spraying reagent: Anisaldehyde in sulphuric acid), R_f 2: retardation factor 2 (spraying reagent: Ferric chloride in 0.5N HCl); Solvent System (Ethyl acetate: methanol: ethanol: water; 81: 11: 4: 8).

the worms.

The main biological activity described for saponins based on recent research is their membrane permeability property. Possible actions of saponins include: changes in membrane permeability and pore formation, similar to the activity of two conventional anthelmintic drugs-praziquantel and toltrazuril. The drugs have been reported to affect the permeability of the cell membrane of worms, causing vacuolization and disintegration of the teguments (Wang, 2010). The present evaluation of

anthelmintic properties of the bulbils of *D. bulbifera* gave data that showed both flesh and peel extracts to possess dose-dependent anthelmintic activity. However, the peel showed stronger anthelmintic activity at 100 mg/ml in destroying worms than that of the flesh. The anthelmintic activity of the peel extracts was more effective on the earthworms, with LC_{50} at 30.40 mg/ml. The anthelmintic activity could be attributed to the presence of trace amount of phenolics and saponins in the plant which may have been responsible for the paralysis and subsequent death of the tested worms. Other medicinal plants where anthelmintic properties attributed to phenolics have been reported include: the root of *Baliospermum montanum* Muell (Euphorbiaceae) and the leaves of *Cassia tora* Linn. (Caesalpinaceae) (Molan et al., 2000; Mali and Wadekar, 2008; John et al., 2009).

Conclusion

This study suggests that flesh and the peel of the bulbils of *D. bulbifera* possess significant anthelmintic property. The present study is the first report on antihelmintic activity of *D. bulbifera* in Nigeria and the ethno-medicinal report of the plant as an anthelmintic drug is confirmed. Efforts shall be aimed at isolating and characterizing the compounds that are responsible for the anthelmintic activity and to establish the mechanism of action.

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Quantitative phytochemical and elemental analysis of *Guiera senegalensis* leaf extract

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Guiera senegalensis **is a shrub found in the savannah region of west and central Africa that is widely used in traditional medicine for the remedy of many ailments/diseases. In this study, some of the phytochemicals and elements present in its leaves were quantified. Alkaloids content was found to be 21.98 g/100 g, saponins 0.28 g/100 g and tannins 0.40 g/100 g. The results showed that alkaloids content is high while saponins and tannins contents are low. The results of the elemental analysis showed that the values of all the elements analyzed compares favorably with values obtained for other plants and thus indicated that *G. senegalensis* leaf contain significant amount of essential mineral elements. Their quantity is in the order Ca > K > P > Na > Mg > Fe > Zn > Cu. The results of this study has justified the widespread usage of *G. senegalensis* leaves as medicine traditionally and also showed that the plant has a lot of potentials in traditional and orthodox medicine.**

Key words: *Guiera senegalensis*, phytochemicals, elements, quantitative analysis.

INTRODUCTION

Guiera senegalensis (Family: Combretaceae) commonly known as Sabara in Hausa is a shrub of the savannah region of west and central Africa. *G. senegalensis* is widely being used in traditional medicine for the remedy of many ailments/diseases. Its leaves extract is being used against dysentery, diarrhea, gastrointestinal pain and disorder, rheumatism and fever (Sule and Mohammed, 2006). In addition, partially purified anthocyanin fraction from leaf extract of *G. senegalensis* has been shown to possess antioxidant property against ccl_4 – induced oxidative stress in rats (Sule and Mohammed, 2009). *G. senegalensis* and *Piliostigma reticulatum* commonly co-exists with crops in the farmer's field throughout the Sahel and their presence can potentially provide more organic inputs to cropped fields than any other source. Traditional management of these shrubs includes coppicing and burning of residues at the beginning of each cropping season, however, non-thermal management of these organic materials hold potential to add organic matter to soils and thus, be a source of nutrients such as nitrogen and phosphorus (Dossa et al., 2009).

G. senegalensis is said to provide ecological benefits to soils and also showed to dramatically increase crop productivity (in some cases 250%) particularly in the Northern Sahel region. Furthermore, it is a locally available resource that can provide crop yield responses even with low or no fertilizer applications, thus making its co-existence with crops in the farm well suited for subsistence, low input farmers (Dossa et al., 2012).

Phytochemicals are bioactive nonnutrient chemical compounds found in plants that work with nutrients and dietary fibre to protect against diseases (Johanna and Jyh-Lurn, 2007; Agbafor and Nwachukwu, 2011). They are secondary metabolites that contribute to flavor and

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color. Many phytochemicals have antioxidant activity and reduce the risk of many diseases (Agbafor and Nwachukwu, 2011). Phytochemicals are many and can be categorized into various groups that is, polyphenols, organosulfur compounds, alkaloids and nitrogen-containing compounds. The polyphenols are some of the most studied compounds and can be further divided into flavonoids (including flavonols, flavones, catechins, flavonones, anthocyanidins and isoflavones), phenolic acids, stilbenes, coumarins and tannins (Johanna and Jyh-Lurn, 2007). Their functions and mechanism of actions may include the following among others: antioxidant activity, hormonal action, stimulation of enzymes, interference with DNA replication and antibacterial properties (Sule and Mohammed, 2009).

However, *G. senegalensis* has been shown to positively contain alkaloids, tannins, flavonoids, amino acids, ascorbic acid, and anthraquinones and also displayed antimicrobial activity (Sule et al., 2002).

Human body requires both metallic and non-metallic elements for healthy growth, development and the proper functioning of the body. Many elements present in the food at major, minor and trace levels are reported to be essential to man's well being. However, their ingestion in excess or limited amount can cause severe health problems (Kumar et al., 2005; Mohammed and Sulaiman, 2009). The determination of these elements in beverages, water, food, plants and soil is thus of utmost importance and is currently the subject of studies by various researchers (Saud and Al-Oud, 2003; Mohammed and Sulaiman, 2009)

MATERIALS AND METHODS

Sample collection and preparation

The leaves of *G. senegalensis* used for this study were collected from a bush around Sule-Tankarkar local government area of Jigawa state, Nigeria. The plant was authenticated at the Botany unit of Bayero University Kano. The leaves were allowed to dry at room temperature and then crushed into powder using laboratory mortar and pestle. The dried powdered leaves were then used for analysis.

Quantitative estimation of alkaloids

Five (5 g) of the dried powdered *G. senegalensis* leaves was extracted with 50 cm^3 of methanol. From the extract, 10 cm^3 was placed in 250 cm^3 separating funnel and 5 cm^3 of dilute H_2SO_4 and distilled water were each added. The extract was shaken twice with 10 cm^3 $CHCl_3$ (trichloromethane) containing 5 cm^3 of dilute H_2SO_4 and 10 cm^3 distilled water. The $CHCl_3$ layer was discarded after shaking and aqueous acidic layer was transferred to the content of first separating funnel. The extract was basified with ammonia solution and was shaken for 30 s. The alkaloids were completely extracted by successive portions of $CHCl_3$. The combined $CHCl_3$ extract was shaken with 5 cm^3 of water and was run through a plug of cotton wool previously moistened with $CHCl_3$. The content was covered with a little anhydrous sodium sulphate which was later washed in 5 cm^3 of $CHCl_3$. The filtrate was then placed into 25 cm^3 conical flask after which the chloroform was distilled completely, followed by the addition of 5 cm^3 neutral alcohol which was evaporated on a boiling water bath. The residue was further heated on boiling water bath for 15 min. The residue was dissolved in 2 cm^3 chloroform and 20 cm^3 N/50 H_2SO_4. The content was warmed to remove $CHCl_3$. The excess acid was titrated with N/50 NaOH using methyl red as indicator, a color change from pink to yellow was observed. The alkaloids contents of the sample were then calculated using the following formula:

Alkaloid content = Amount (cm^3) of N/50 NaOH × 0.005787 × 100 / 10 (g% w/v) according to Wasagu et al. (2005).

Quantitative determination of tannins

Five (5 g) of dried powdered *G. senegalensis* leaves were macerated in distilled water and allowed to stand overnight. The water extract (5 cm^3) was placed in a stoppered conical flask. A quantity (25 cm^3) of 0.1 N iodine and 10 cm^3 of 4% NaOH were added. The resulting mixture was kept in the dark for 15 min. Water (10 cm^3) was used to dilute the mixture and was acidified with 10 cm^3 4% H_2SO_4. The mixture was titrated with 0.1 N sodium thiosulphate solution and starch was used as indicator. The titration value corresponded to the sum of tannins and pseudo tannins-concentration A. Another 25 cm^3 of the water extract was placed in a stoppered conical flask followed by 15 cm^3 of 1% gelatin. The volume was made up with distilled water and filtered. Aliquot of 20 cm^3 was placed in a volumetric flask; 25 cm^3 of 0.1 N iodine and 10 cm^3 of 4% NaOH were added, mixed and kept in the dark for 15 min. The mixture was diluted with 10 cm^3 water and acidified with 10 cm^3 4% H_2SO_4. This was finally titrated with 0.1 N sodium thiosulphate using starch solution as indicator. The titration value obtained corresponded only to pseudo tannins - concentration B. A blank experiment was carried out simultaneously using distilled water. The tannins and pseudo tannins contents of the sample were then calculated using the following formula:

% of Pseudo tannins = Blank Expt. × 0.029 × 100 / 5

% of True tannins = A – B (g% w/v) according to Wasagu et al. (2005).

Quantification of saponins

Five (5 g) of dried powdered *G. senegalensis* leaves were macerated in methanol allowed to stand overnight. The mixture was then filtered to obtain precipitate that is, methanolic extract which was allowed to dry. The dried methanolic extract was then dissolved in water and partitioned with an equal volume of n – butanol. The n – butanol fraction was then collected and the aqueous layer was discarded. The n – butanol fraction was concentrated and dried. It was then dissolved in methanol and diethyl ether was then added drop wise to precipitate the saponins. The mixture was filtered with a pre-weighed filter paper. The precipitate corresponds to the quantity of the extracted saponins (Wasagu et al., 2005).

Elemental analysis

A 0.5 g of dried powdered *G. senegalensis* leaves were digested using 10 cm^3 of a mixture of concentrated HNO_3 and concentrated

HCl (3:1 v/v). Analytical grade reagents were used for the preparation of the standard solutions of these elements (Ca, Zn, Mg, Fe, Cu, Na, K, and P). The diluted digests were analyzed using atomic absorption spectrophotometer (AAS) for Ca, Cu, Fe, Mg and Zn, while flame photometer was used for Na, K and P.

RESULTS AND DISCUSSIONS

The results of this study are presented in Tables 1 and 2. Table 1 show the results of quantitative phytochemical analysis of *G. senegalensis* (GS) leaf extract and the result showed high content of alkaloids, low concentration of saponins and tannins. Table 2 shows the concentration of mineral elements determined for GS leaf extract. The results showed that potassium has the highest concentration while copper has the least concentration among the mineral elements analyzed.

The high content of alkaloids in GS leaf extract agrees with earlier reports that alkaloids concentration decreased in the roots with corresponding increase in the foliar parts, which suggest that alkaloids are translocated from the roots upward to the leaves and stems (Ralph and Gardner, 2003). The saponins and tannins content are relatively low when compared with values obtained from other plants; 0.386 and 0.456%, respectively (Soliz-Guerrero et al., 2002). The natural plant products that have received greatest attention with regards to possible medicinal application are the alkaloids and saponins. In addition, alkaloids and flavonoids were also reported to be responsible for antimicrobial properties in some ethno medicinal plants (Singh and Bhat, 2003). Furthermore, many tannin molecules have also been shown to reduce the mutagenic activity of a number of mutagens. Many carcinogens and/or mutagens produce oxygen free radicals for interaction with cellular macromolecules. The anticarcigenic and antimutagenic potentials of tannins may be related to their ant oxidative property, which is important in protecting cellular oxidative damage including lipid per oxidation (Chung et al., 1998).

Table 2 shows the results of mineral content determined for the GS leaf. Calcium which is the most common mineral element in the body helps in the transport of long chain fatty acids which aid in prevention of diseases, high blood pressure and other cardiovascular diseases. The results of calcium analysis obtained 8882.492 ppm (Table 2) were higher than those reported by Oladele and Oshodi (2007) and also higher than that reported by Mohammed and Sulaiman (2009). Copper is essential to all living organisms as a trace dietary mineral element. It is a key constituent of the respiratory enzyme complex cytochrome c oxidase which is required in aerobic respiration. Copper is also a component of the protein hemocyanin which is the oxygen carrier in most mollusks and arthropods. The results obtained for copper are lower than values reported by Aderibigbe and Brown (1993) but higher than that reported by Tokusoglu and Unal (2003) and Saud and Al-Qud (2003).

Table 1. Results of quantitative phytochemical analysis of GS leaf extract.

Phytochemical	Quantitative value (g 100^{-1} g)
Alkaloids	21.98
Tannins	0.28
Saponins	0.40

Table 2. Results of elemental analysis of GS leaf extract.

Element	Concentration (ppm)
Ca	219.03
Cu	19.80
Fe	497.36
K	5200.00
Mg	1315.57
Na	1400.00
P	1808.31
Zn	43.70

Iron (Fe) is a necessary trace element found in nearly all living organisms. It plays an important role in biology, forming complexes with molecular oxygen in hemoglobin and myoglobin which are oxygen transport proteins in vertebrates. Many enzymes vital to life also contain iron, such as catalase and lipoxygenase. The color of blood is also due to iron containing hemoglobin. The values obtained are lower than that reported by Aderibigbe and Brown (1993) for water hyacinth and water lettuce and also lower than that reported by Tokusoglu and Unal (2003) for chlorella and isochrisis algae. However, the obtained result for iron is higher than (3.39 ± 0.52 mg 100^{-1} g) reported by Oladele and Oshodi (2003) for *Jatropa cathartica* seeds and also higher than (3.19 mg dm^{-3}) reported by Mohammed and Sulaiman (2009) for tea leaf samples.

Potassium is important for reducing blood pressure and also increasing blood circulation as well as preventive aid on general health. The results in Table 2 shows the level of potassium in GS leaf which is generally lower than (987.48 ± 2.13 mg 100^{-1} g) reported by Oladele and Oshodi (2007) for *Jatropa cathartica* seeds. The result is higher than (33.1 mg dm^{-3}) reported by Mohammed and Sulaiman (2009) for tea leaf sample. Magnesium is an essential mineral element in biological systems. It is present in every cell type in every organism. ATP (adenosine triphosphate) the main source of energy in cells must be bound to a magnesium ion in order to be biologically active. Over 300 enzymes require the presence of Mg^{2+} for their catalytic action including all

enzymes utilizing or synthesizing ATP or those that use other nucleotides to synthesize DNA and RNA. In plants, magnesium is necessary for synthesis of chlorophyll and photosynthesis. The values obtained for magnesium (1315.570 ppm) are lower than that reported by Oladele and Oshodi (2007) and are higher than values obtained by Mohammed and Sulaiman (2009).

Sodium is an essential mineral element in humans that regulates blood volume, blood pressure, osmotic equilibrium and pH. Thus, it is the major cat ion in blood and extracellular fluid (ECF). In plants, sodium is a micronutrient that aids in metabolism, specifically in regeneration of phosphoenolpyruvate and synthesis of chlorophyll. The results obtained for sodium are lower than values reported by Aderibigbe and Brown (1993) for water hyacinth and water lettuce, also lower than values obtained for three microalgae by Tokusoglu and Unal (2003). However, the results are higher than that obtained for *J. cathartica* seeds by Oladele and Oshodi (2007).

The results obtained for phosphorus 1808.31 ppm (Table 2) are lower than (2125.19 ± 0.00 mg 100^{-1} g) for *J. cathartica* seed reported by Oladele and Oshodi (2007) phosphorus in GS leaf is low. However, phosphorus is also essential for all forms of life. As phosphate, it is a component of DNA, RNA, ATP and also the phospholipids that form all cell membranes. It plays a major role in DNA and RNA where it forms part of the structural framework of these molecules. Living cells uses phosphorus as phosphate to transport cellular energy in the form of ATP. ATP is important in phosphorylation, a key regulatory event in cells. Zinc is an essential mineral element of exceptional biologic and public health importance necessary for plants, animals and micro-organisms. It is important in metabolic function and for growth in man. Zinc is also found in nearly 100 specific enzymes. Enzymes with zinc atom in the reactive center are widespread in biochemistry such as alcohol dehydrogenase in humans. The concentration of zinc in plants varies based on levels of the element in soil. Table 2 shows the level of zinc in GS leaf (43.700 ppm). The results obtained are generally higher than (2.17 mg dm^{-3}) reported by Mohammed and Sulaiman (2009) and that reported by Tokusoglu and Unal (2003) for three microalgae. The values are lower than (47.22 ± 0.24 mg 100^{-1} g) reported by Oladele and Oshodi (2007) for *J. cathartica* seeds.

Conclusion

The results obtained have shown that GS leaf has high concentration of alkaloids and low concentration of tannins and saponins. The results of the phytochemical content have therefore justified the widespread usage of the plant as medicine traditionally. The results of mineral elements analyzed for GS leaf compares favorably with other values obtained by previous researchers and thus indicated that the leaf contain significant amount of the mineral elements. It could therefore be concluded that GS leaf is a potential source of active ingredients that could be used in both traditional and orthodox medicine and that it is also a source of essential mineral elements.

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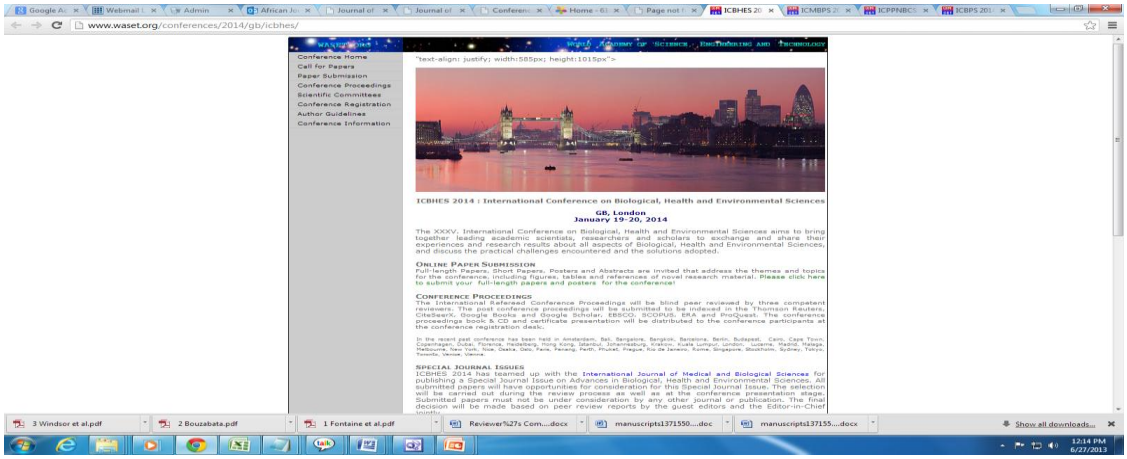

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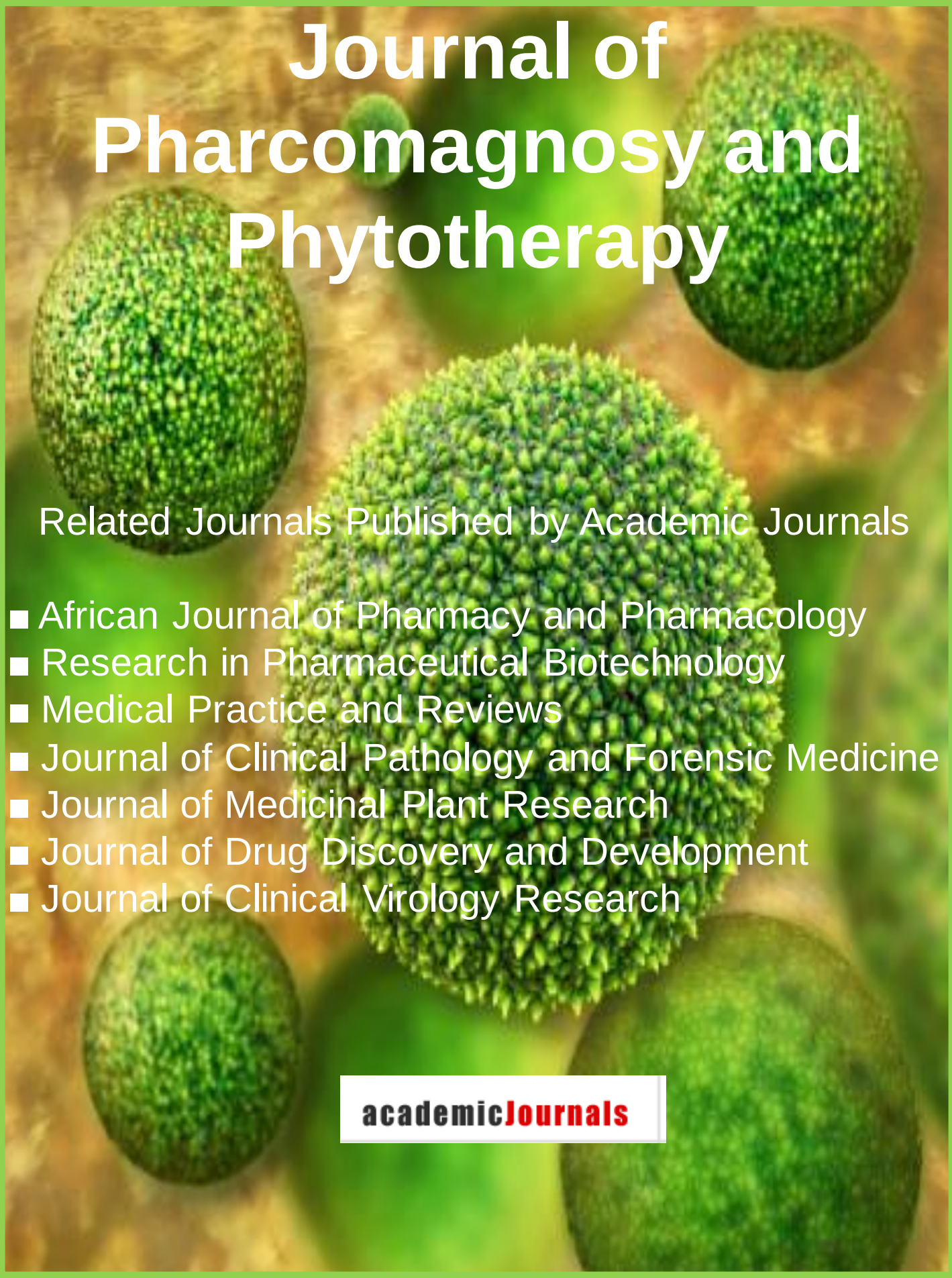

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