Journal of
Plant Biochemistry & Physiology

Shafei et al., J Plant Biochem Physiol 2016, 4:2
DOI: 10.4172/2329-9029.1000166

Research Article Open Access

Antitoxic, Antifungal and Phytochemical Analysis of Medicinal Compounds of *Guiera senegalensis* Leaves in Sudan

Nabaa Kamal Al Shafei[1]*, Abdulkadir Elzein Elshafie[2] and Abdelfattah Nour[3]

[1]Department of Biochemistry, Faculty of Veterinary Medicine, University of Bahri, Sudan
[2]Department of Biology, College of Science, Sultan Qaboos University, Oman
[3]Department of Basic Medical Sciences, College of Veterinary Medicine, Purdue University, USA

Abstract

Guiera senegalensis (*Gs*) is a well-known traditional medicinal plant in Africa, whose leaves extract and roots powder are used for treatment of diseases and wounds in Western Kordufan, Sudan. The aim of this study was to investigate the phytochemical analysis, toxicity, and the antifungal activity of *Gs* leaves extract. Extract of the leaves of *Gs* was tested for its antimicrobial activity against *Stemphylium solani, Aspergillus flavus, Trichoderma viride, Penicillium* Spp., *Fusarium verticillatum, Cladosporium cladosporioides, and Fusarium solani*. This present study showed that *Gs* leaves extract has no inhibition activity against all of the tested fungal strains. On the other hand, the toxicity test, which was conducted by using brine shrimp, suggests that *Gs* leaves extract is apparently not toxic. The phytochemical screening revealed that *Gs* leave extract contains alkaloids, flavonoids, terpenoids, tannin, carbohydrates, proteins, steroids, and saponins. The results of this preliminary investigation suggests that the medicinal plant extract may be safe to use as a drink for treatment of various diseases as has been practiced for years in the villages of Western Sudan. More research is needed to investigate if there is any side effect when the extract is taken orally. Further, the medicinal properties of the phytochemical compounds of *Gs* need to be further investigated.

Keywords: *Guiera senegalensis*; Antifungal activity; Brine shrimp toxicity; Phytochemical analysis; Sudan

Introduction

Several traditional medicinal plants, including *Guiera senegalensis (Gs)*, a shrub that grows well in sub-Saharan Africa and Sudan [1], have been candidates for research because of their perceived medicinal properties. Evaluation of compounds such as, tannins, alkaloids, flavonoids saponins, terpenoids and phenols have been used as a method of screening of medicinal plants [2]. *Gs* has been used in Western Kourdofan of Sudan and elsewhere in traditional medicine as a cure for infections and wounds [3,4]. In the Sudan, *Gs* is locally known as Ghubaysh of which the leaves extract and the roots powder are used for treatment of a variety and diseases and wounds, respectively. In a companion paper [4], we found that *Gs* leaves' extract has been used for treating jaundice which represents more than 51.5% of conditions treated; and 48.5% of the other diseases that include diabetes mellitus, hypertension, cough, arthritis, enteritis, diarrhea and malaria. In addition, the majority of people surveyed have used roots' powder of *Gs* for treatment of wounds, including diabetics wounds, and inflammation of skin, and injuries. *Gs* extract was also used in the folk medicine in other countries and found to be effective against leprosy, fever, and was helpful against increased blood pressure and high blood sugar levels [5]. In addition to its usefulness as traditional medicinal plant, *Gs* has been shown to increase soil fertility and crop production without use of fertilizers in in the Northern Sahel region, especially in low-yields-low input farms [6]. The importance of *Gs* in traditional medicine became more apparent with the recent increase in fungal infections in Africa, and elsewhere. Extracts of Leaves, shoots and galls of *Gs* were found to be useful against bacteria and fungi infections in Burkinabe folk medicine [7]. These antimicrobial properties were credited to the crude methanolic extracts of *Gs* [8]. Studies of medicinal plants usually concentrate on the part of the plant that has been shown to have value in traditional medicine. Although *Gs* leave extract has been used to treat jaundice and many diseases in Western part of Sudan, the antifungal activity of leave extract has not been documented. The plant extract is prepared by boiling the leaves or soaking them in water [4]. Since there are thousands of species of fungi present in the environment and at lease a hundred of them are pathogenic [9], antifungal activity of medicinal plants needs to be addressed. It worth mentioning that the antifungal activity of many medicinal plant species in Africa has not been satisfactorily explored. The aim of study was to examine the phytochemical properties, antimicrobial efficacy and toxicity of *Gs* leaves' extract in order to find out their biological activity.

Materials and Methods

Plant materials

The leaves of *Gs* used in this study were randomly collected from *Gs* bushes around Ghubaysh village area of Western Kordufan State, West of the Sudan. The plant leaves were dried in the shade. After drying, the leaves were ground well into fine powder using mechanical blender, and the powder was transferred into airtight containers with proper labeling for future use.

Water extraction

Five grams of the plant powder was placed in a beaker containing 100 ml. distilled water; soaked and shaken well. The solution was filtered with the help of filter paper and the filtrate was kept and used for further phytochemical analysis. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use.

***Corresponding author:** Nabaa Kamal Al Shafei, Department of Biochemistry, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan, Tel: +249122410042; E-mail: nabaak@yahoo.com

Received March 11, 2016; **Accepted** March 30, 2016; **Published** April 07, 2016

Citation: Shafei NKA, Elshafie AE, Nour A (2016) Antitoxic, Antifungal and Phytochemical Analysis of Medicinal Compounds of *Guiera senegalensis* Leaves in Sudan. J Plant Biochem Physiol 4: 166. doi:10.4172/2329-9029.1000166

Toxicity test

Brine shrimp (Artemia) lethality bioassay, was carried out to investigate the cytotoxicity of plant extract. Fifty mg of Artemia salina (Leach) eggs were added to a hatching chamber containing sea salt\ water (30 gr\L). The hatching chamber was kept under an inflorescent bulb for 48 h for the eggs to hatch into shrimp larvae. Different concentration of the extract (250, 500 and 1000) µg in 40 ml distilled water were prepared in triplicate vials. Ten brine shrimp larvae were then placed in each of the three duplicate vials. Others were placed in distilled water to serve as a negative control and incubated for 24 h. After 24 h the nauplii were examined against a lighted background, with a magnifying glass and the average number of survived larvae was determined [10].

Fungal mycoflora

One mg of plant powder under investigation was cultured in each one of the seven petri dishes containing Potato Dextrose agar (PDA) and incubated at room temperature for seven days.

Antifungal test

Antimicrobial activity testing was carried out by using agar spread method. One ml of the *Gs* extract (5%) was spread to each one of seven (PDA) that were cultured with Stemphylium *solani, Aspergillus flavus, Trichoderma viride, Penicillium* spp*, Fusarium ver ticillatum, Cladosporium cladosporioides, Fusarium solani.* The petri dishes were incubated for seven days at room temperature. At the end of incubation, zone of inhibition that developed were measured with a ruler. Distilled water was used as a control.

Test for flavonoids

For the confirmation of flavonoids in the *Gs* leave powder, 0.5 g of plant extract was placed in a test tube and 10 ml of distill water was added, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of the plant extract followed by addition of 1 ml concentrated H_2SO_4. Indication of a yellow color shows the presence of flavonoids in each extract [11].

Test for alkaloids

The procedure used for determination of alkalolids in *Gs* sample is similar to that described by [11]. Briefly, 0.2 g of *Gs* leave powder was added to a glass test tube that has 3 ml of hexane. The powder and hexane were thoroughly mixed, shaken well, and filtered. To the hexane and *Gs* leaves extract mixture, 5 ml of 2% HCl were added, and the mixture heated until boiling. The mixture was then filtered, and 1-3 drops of picric acid were added to the hexane, HCl and *Gs* leave extract filtrate. The presence of alkaloids in the *Gs* sample was confirmed by the yellow- colored precipitate that was formed.

Test for terpenoids

An amount of 0.8 gram of the *Gs* leave powder was placed in a test tube, then 10 ml. of methanol was poured in it, shaken well and filtered. Five ml of the plant extract of plant sample was taken. Then 2 ml of chloroform were mixed with the extract and 3 ml of sulphuric acid were added. Formation of reddish brown color indicates the presence of terpenoids in the selected plants [11].

Test for tannins

Crude extract of *Gs* powder was mixed with 2 ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of tannins [12].

Test for proteins, ninhydrin test

The Crude extract of *GS* leaves powder was boiled with 2 ml of 0.2% solution of Ninhydrin. Violet color appearance will suggest the presence of amino acids and proteins [12].

Test for carbohydrates, Fehling's test

Two ml. of equal volumes of Fehling A and Fehling B reagents mixed together were added to the crude extract and gently boiled. A brick red precipitate appearing at the bottom of the test tube indicates the presence of reducing sugars [12].

Test for steroids

Gs leaves powder was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2 ml of chloroform. Then 2 ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids [12].

Test for saponins

The crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins [12].

Results and Discussion

Toxicity test

In the present study**,** brine shrimp (artemia) toxicity test was used to find out if the leaves extract is safe to consume. The artemia toxicity test is used routinely because it convenient to use in addition to being inexpensive. As a routine test, it is employed as an initial step in the assessment and evaluation of the toxic characteristics of a substance [13], for screening drugs, and for determination of lethal doses, and for assessing health hazards that may arise from short term exposure to drugs [14]. On these grounds, we decided to use the brine shrimp (artemia) toxicity test to evaluate if the traditional medicinal plants *Gs* leave extract has potential toxicity. In the present study, the leave extracts from *Gs* showed no toxicity to brine shrimps at concentrations of 250,500, and 1000 µg in 40 ml of distilled water, and that was confirmed by the number of dead brine shrimp larvae, which was zero. From this result, we can conclude that the *Gs* leaves extract is apparently not toxic. Our result are in general agreement with that obtained by Ref. [14] who observed that water extract of *Gs* given at the rate of 2 g/kg to Wister male and female rats showed no toxicity as confirmed by the evaluation of pathological changes in different organs, hematological parameters, and urinalysis. The results of the present study are also in agreement with Ref. [15] who concluded that *Gs* aqueous leave extract is not toxic and may be safe, especially at the dose used for treatment, which is greatly lower than the doses used in their experiments. However, the findings of the present study are not in agreement with that found by Ref. [16] who reported signs of toxicity when experimental animals were drenched with the leaves powder of *Gs.* At the dosage of 1 or 2 g/k/day**.** This disagreement could be explained by the fact that the present study used the aqueous extract of the leaves while Ref. [16] used the actual leaves powder. Interestingly, Ref. [16] observed that the treated animals became drowsy suggesting an effect of *Gs* powder on the cardiovascular system function and blood pressure. This may explain the folk use of the plant for hypertension control. Further, Ref. [17] reported that spontaneous motor activity

was decreased in mice by administration of the aqueous extract of *Gs*, and that the sleeping time induced by pentobarbital was prolonged in treated mice.

Fungal test

Physical and microscopic examination of the cultured *Gs* powder showed growth of Aureobasidium pullulans, *Penicillium* spp. and Rhizopus (Figure 1).

Antifungal activity

In developing countries, medicinal plants are gaining popularity because of their perceived effects against microbes, and cost or invariability of health care. The World Health Organization (WHO) reported that approximately 80% of the world population use traditional medicinal plants the extracts or the active ingredients in medicinal plants [18]. On the other hand, more than 60% of the successful drugs developed against infections during the last twenty years utilized natural products [19]. Therefore, the interest in traditional medicinal plants will continue to grow as emerging infectious diseases continue to expand, and the numbers of drug resistant microbes continue to escalate. Further, plant extract are often consumed without paying attention to safety issues. In the present work, the antimicrobial activity of the extracts of *Gs* were studied against seven fungal strains. For the antifungal susceptibility test, the leaves extracts obtained from *Gs* showed no activity (No inhibition) against all of the tested fungal strain. Ref. [20] indicated that since solvents used for extraction are expected to have different range of solubility for the phytochemical component of the medicinal plant, ethanolic extract would be more effective in treatment than an aqueous extract. In Western Sudan, people use the aqueous extract of the leaves as a medicine. However, from drug discovery point of view, it would be interesting to quantify antimicrobial activity of *Gs* ingredients extracted using ethanolic solvent in which plant material is expected to dissolve better.

Phytochemical analysis

This study also showed the presence of different phytochemicals whose biological activity can be of valuable therapeutic index. The result of the presence of the phytochemicals in *Gs* extract, shown in Table 1, is indicated by the colour intensity in a scale of 4 (+, ++, +++, ++++).

Since medicinal plants, such as *Gs*, are expected to be a valuable source for a variety of drugs, no wonder that 80% of individuals from developed countries use traditional medicines extracted from plants according to WHO [18]. It, therefore, is important to investigate traditional medicinal plants to assess, following scientific methodologies, their properties, safety, and efficiency of treatment, and the optimum dose [21]. Table 1 shows that plant under investigation contains flavonoids, alkaloids, terpenoids, tannin, proteins, carbohydrates, steroids and saponins. Our results are in the same line with the findings obtained by Ref. [22] who indicated that *Gs* leave extract had been found to contain alkaloids, tannins, flavonoids, amino acids, in addition to its antimicrobial activity. The results of the present study also agrees with that reported by Ref. [23] who also confirmed the presence in *Gs* leave extract of flavonoids, saponins, tannins, terpenes, and carbohydrates. One of ingredients that we found in *Gs* leave extract are terpenoids, which are found to have a variety of pharmacological activities such anti-bacterial, anti-inflammatory and anti-malarial activities [24,25]. The presence of flavonoids in *Gs* leaves extract documented in this study supports the findings of Ref. [26] who stated that the antimicrobial properties in some traditional medicinal plants can be due to the presence of flavonoids. Furthermore, several biological effects such as antioxidants abilities, and anti-inflammatory effects are attributed to flavonoids and phenolic compounds in medicinal plants [27]. These observations and the findings of the present study suggest there is an urgent need for investigating why *Gs* extract is credited for curing several diseases and infections in Africa. It would important to find out which phytochemical compound in *Gs* is responsible for what activity.

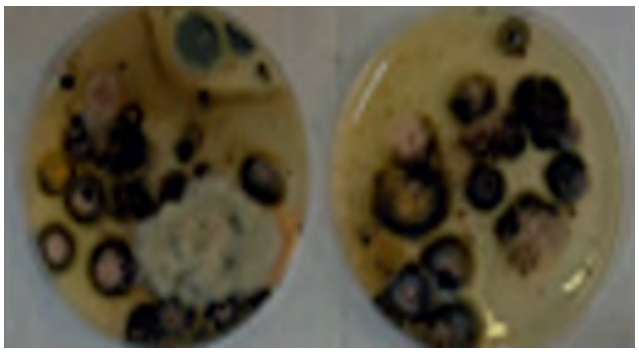

Figure 1: Growth of fungi in in Potato Dextrose agar.

The content of alkaloids, tannin and saponins found in the present study agree with that reported by Ref. [28] who reported high levels of alkaloids and low tannins and saponins content in *Gs*. Alkaloids found in medicinal plants used as anesthetic agents [29], a premise that was not investigated in the present study. In addition, tannin have been found by Ref. [30] to have a wide range of antimicrobial and anti-inflammatory effects. The anticarcinogenic potentials of tannins could be attributed to their antioxidative properties that protect living cells from oxidative damage [31]. It is documented in this study that steroids were present in *Gs* leave extract. These steroids have antibacterial effects [32], and are important because of its relationship with steroid hormones such as sex hormones estrogen and testosterone [33].

The observed antibacterial effects of the medicinal plants extracts have been related to the presence of tannins, flavonoids and saponins [34]. This is in agreement with Ref. [35] who investigated the effect Eucalyptus *Camaldulensis* extract against pathogenic bacteria (*Salmonella typhi* and *Escherichia coli)* and attributed the effect of the plant extract to the active phytochemical compounds it has, namely tannins, flavonoids and saponins. *Gs* has been shown to contain compounds such as alkaloids, tannins, flavonoids that demonstrated their antimicrobial activity [36].

Conclusion

In conclusion, medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs for treatment of various diseases. The results obtained in the present study have shown that *Gs* leaves have high concentration of alkaloids and low concentration of tannins and saponins and have no toxic effect and show no antimicrobial activities in case of fungi. These observations and the findings of the present study suggest an urgent need for investigating why *Gs* extract is credited for curing several disease and infections in Africa. It would important to find out which phytochemical compound in *Gs* is responsible for what activity. Thus, we hope that the important phytochemical compounds identified by our study in the *Gs*, will be helpful in treating different diseases of this particular region of Africa, and may be in other regions. The plant extract may be safe, especially at the therapeutic dose which

Plant species	Flavonoids	Alkaloids	Terpenoids	Tannin	Proteins	Carbohydrates	Steroids	Saponin
Guiera senegalensis	+++	++++	++	+	++	++	++	+

+=indicates presence of phytochemicals; ++=indicates small presence; +++=indicates moderate presence; ++++=indicates High presence

Table 1: Phytochemical component of *Guiera senegalensis* leaf extract.

is far lower than the tested doses. The results could serve for further pharmacological and phytochemical research. Also more research needed to evaluate the potential effectiveness of the crude extracts as the antimicrobial agents.

Acknowledgments

The authors would like to thank Bashir Ibrahim Alblaa for his kind help in collecting samples from the field.

References

1. Ames BN, Gold LS, Willet WC (1995) The causes and prevention of cancer. Proc. Natl Acad Sci 92: 5258-5265.
2. Hill AF (1952) Economic Botany. A textbook of useful plants and plant products. New York: McGraw-Hill Book Company Inc.
3. Sule MS, Mohammed SY (2006) Toxicological studies on the leaves of Guiera senegalensis and Psidium guajava in rats. Biol Environ Sci J Trop 3: 81-83.
4. Alshafei NK, Ahmed SM, Abdelfattah NA (2016) Preliminary Observations on the Uses of *Guiera Senegalensis* as a Traditional Medicinal Plants in Western Kurdofan, Sudan. Journal of Plant Biochemistry and Physiology.
5. El-Gazali GEB, El-Tohami MS, El-Egami AAB (1994) Medicinal Plants of the Sudan: Medicinal Plants of the White Nile Province. Medicinal and Aromatic Plant Research Institute, Khartoum Sudan.
6. Dossa EL, Diedhiou I, Khouma M, Sene M, Lufafa A, et al. (2012) Crop Productivity and Nutrient Dynamics in a Shrub Guiera senegalensis-Based Farming System of the Sahel. Agron J 104: 1255-1264.
7. Nacoulma OG (1996) Plantes médicinales et Pratiques médicales Traditionnelles au Burkina Faso. cas du plateau central T1 and T2. Thèse Doctorat d'Etat ès Sciences Naturelles. Université de Ouagadougou.
8. Bassene E, Mahamat B, Lo M, Boye CSB, Faye B (1995) Comparaison de l'activité antibactérienne de trois Combretaceae. Combretum micranthum, Guiera senegalensis, Terminalia avicennioides. Fitoterapia 66: 86-87.
9. Keeler RF, Tu AT (1991) Toxicology of Plant and Fungal Compounds. Handbook of Natural Toxins 6: 665.
10. Mayer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nicholas PE, et al. (1982) Brine shrimp, a convenient general bioassay for active plant constituents. Planta Medica 45: 31-34.
11. Wadood A, Mehreen G, Syed BJ, Muhammad N, Ajmal K, et al. (2013) Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. Biochem Anal Biochem 2: 144.
12. Yadav RNS, Agarwala M (2011) Phytochemical analysis of some medicinal plants. Journal of Phytology 3: 2075-6240.
13. Tarzwell CM (1969) Standard Methods for Determination of Relative Toxicity Of Oil Dispersants And Mixtures Of Dispersants And Various Oils To Aquatic Organisms. International Oil Spill Conference Proceedings 1: 179-186.
14. Akhila JS, Deepa S, Alwar MC (2007) Acute toxicity studies and determination of median lethal dose. Curr. Sci 93: 917.
15. Diouf A, Cisse A, Gueye SS, Mendes V, Siby T, et al. (2000) Toxocological study of Guiera senegalensis Lam (Combretaceae). Dakar Med 45: 89-94.
16. Hauwa'u YB, Maryam I, Ja'afaru SM, Maimuna Z, Timothy B (2014) Toxicity studies of aqueous, methanolic and hexane leaf extracts of Guiera senegalensis in rats. International Journal of Scientific and Engineering Researchl 5: 1339.
17. Ilham MO, Adam SEI, Mohammed AS (2011) Toxicological Effects of *Guiera senegalensis* Family: *Combrataceae* on Nubian goats. The Sudan J Vet Res 26: 65-70.
18. Amos S, Kolawole E, Akah P, Wambebe C, Gamaniel K (2001) Behavioural effects of the aqueous extract of Guiera senegalensis in mice and rats. Phytomedicine 8: 356-361.
19. Shaik D, Malika FA, Rafi SM, Naqui B (1994) Studies of antibacterial activity of ethanolic extract from Nericum indicum and Hibiscus rosasinensis. J Islamic Acad Sci 7: 167-8.
20. Cragg GM, Newman DJ (2005) Biodiversity: A continuing source of novel drug leads. Pure Appl Chem 77: 7-24.
21. Srinivasan D, Perumalsamy LP, Nathan S, Sure T (2001) Antimicrobial Activity of Certain Indian Medicinal Plants Used in Folkloric Medicine. Journal Ethnophatmacol 94: 217-222.
22. Arunkumar S, Muthuselvam M (2009) Analysis of phytochemical constituents and antimicrobial activities of aloevera L. against clinical pathogens. World J Agri Sc 5: 572.
23. Sule AM, Thanni LOA, Sule OOA (2002) Bacterial pathogens associated with infected wounds in Ogun State University Teaching Hospital, Sagamu, Nigeria. African Journal of Clinical and Experimental Microbiology 3: 13-16.
24. Staden JV, Grobbelaar N (1995) The effect of Sesbanimide and *Sesbania* seed extracts on germination and seedling growth of a number of plant species. Journal of Environmental and Experimental Botany 35: 321-325.
25. Mahato SB, Sen S (1997) Advances in triterpenoid research 1990-1994. Phytochemistry 44: 1185-236.
26. Singh B, Bhat TK (2003) Potential Therapeutic Applications of some Antinutritional Plant Secondary Metabolites. J Agric Food Chem 51: 5579-5597.
27. Asha K, Rasika CT, Nirmala RD, Jyoti PS (2011) Antioxidant Potential from Stem Bark of Juglans regia L. Ann. Biol Res 2: 176-180.
28. Mohammed SY (2013) Quantitative phytochemical and elemental analysis of Guiera senegalensis leaf extract. Journal of Pharmacognosy and Phytotherapy 5: 204-207.
29. Hérouart D, Sangwan RS, Fliniaux MA, Sangwan NBS (1988) Variations in the Leaf Alkaloid Content of Androgenic Diploid Plants of Datura innoxia. Planta Med 54: 14-17.
30. Lata N, Dubey V (2010) Preliminary phytochemical screening of Eichhornia crassipes: the world's worst aquatic weed. J of Pharmacy Research 3: 1240-1242.
31. Chung KT, Wong TY, Wei CL, Huang YW, Lin Y (1998) Tannins and human health: a review. Crit Rev Food Sci Nutr 38: 421-464.
32. Raquel FE (2007) Bacterial lipid composition and antimicrobial efficacy of cationic steroid coppounds. Biochemica et Biophysica Acta, pp: 2500-2509.
33. Okwu DE (2001) Evaluation of chemical composition of medicinal plants belonging to Euphorbiaceae. Pak Vet J 14: 160-162.
34. Osadebe PO, Okide GB, Akabogu IC (2004) Study on the anti-diabetic activity of crude methanolic extract of Loranthus micranthus Linn. sourced from five different host trees. J Ethnopharmacology 95: 133-138.
35. Lutterodt GD, Ismail A, Basheer RH, Baharudin HM (1999) Antimicrobial effects of *Psidum guajava* extracts as one Mechanism of its Antimicrobial action on Escherichia coli and Salmonella typhi. Malaysia Journal Medical Science 6: 17-20.
36. Sule MS, Bichi LA, Atiku MK (2001) Antimicrobial and Preliminary Phytochemical Screening of Guiera senegalensis, Euphorbia lateriflora and Mitracapus scaber. W Afr J Pharmacol Drug Res 18: 12-13.