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Phytochemical and Antimicrobial Screening of Some Commonly Consumed Herbal Medicines in Kano State, Nigeria.

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Abstract: *Five unregistered herbal medicinal concussions commonly used in Kano Metropolis, Kano State, Nigeria, comprising of remedy for pile (MB), yellow fever (MS), typhoid (MT), stomach pain (MC) and sexually transmitted diseases (STDs) were evaluated for their phytochemicals and antimicrobial activity using standard methods. Three different solvents (pet-ether, chloroform and ethanol) were used for the extraction. The results of the phytochemical screening showed the presence of Saponin, Tannin, Terpenoids, Flavonoid, Reducing sugar, Alkaloids and Anthraquinone in most of the extracted samples. Amino acid was absent in all the samples. The antimicrobial activity of these medications was studied against four bacterial clinical isolates (S. aureus, E. coli, Shigella, and Salmonella) bacteria using Agar Well diffusion technique. The most effective antibacterial activity was shown by Ethanolic extracts of sample MS (20mm zone of inhibition) and sample MT (19mm zone of inhibition) against Salmonella typhi, compared to the standard antibiotic Ciprofloxacin (32mm zone of inhibition). Minimum inhibitory concentration (MIC) of the extracts showed chloroform extract of sample MTS to have the lowest inhibitory concentration of 125μg/ml on Staphylococcus aureus. The Minimum bactericidal concentration (MBC) of all the extracts exceeded 1000μg/ml. From the results of the phytochemical and antimicrobial screening, it showed that the Medicinal plants/products could be used as potential source of antibacterial agents.*

Keywords: *Phytochemicals, Antimicrobial, Herbal medicines, Kano State.*

INTRODUCTION

Herbal medicine is defined as a plant derived material or preparation with therapeutic or other human health benefits, which contains either raw or processed ingredients from a single or multiple plants. Herbal medicines are generally regarded as safe based on their long-standing use in various cultures. However, there are reports of serious adverse effects after administration of herbal products. In some of the cases, the toxicity has been traced to contaminants and adulterations.Herbal medicines play significant roles in the management of both minor and major illnesses and have been influenced by patients' dissatisfaction with conventional allopathic medicines in terms of effectiveness, safety and satisfaction with therapeutic outcome (Huxtable, 1990; Abbot, 1997; Barnes, 2003). Some of the more complex reasons for preference of herbal medicines are associated with cultural and personal beliefs (Ernst, 2000).

It has been known in the literature that many plants have medicinal values. This property is due to the presence of active components generally referred to as phytochemicals. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties and are reported to cure ailments such as cancer, heart attack, and stroke among others. They are non-essential nutrients, because the body does not require them for sustaining life. There are more than a thousand known phytochemicals, which are classified as alkaloids, Terpenoids, flavonoids, Saponin, tannins, anthraquinone, glycosides, cardiac glycosides, carbohydrates etc. These vast numbers of natural compounds have diverse antimicrobial potential and are products of secondary metabolites (Trease and Evans, 2002).

Alkaloids: Alkaloids are a naturally occurring large group of pharmacologically active nitrogen-containing secondary metabolites of plants, of microbial or animal origin. Besides being poisonous, the primary function of alkaloids in all vegetation is to protect them from grazing animals and herbivorous insects.

Glycosides

Glycosides are compounds that yield one or more sugars upon hydrolysis. It is composed of a sugar potion (glycones) and non-sugar portion (aglycones or genin). Glycosides of many different aglycones are extensively found in the plant kingdom.

Flavonoids

The flavonoids are a large group of natural products, which are widespread in higher plants but also found in some lower plants, including algae. Most flavonoids are yellow compounds, and contribute to the yellow color of the flower and fruits, where they are usually present as glycosides.

Tannins

Tannins are a heterogeneous group of natural products widely distributed in the plant kingdom and derived its name from the technical word 'tanning' that meant converting animal hides to leather through chemical processes; tannin is basically used for this function. They are polyphenol found in abundance in the tree bark, wood, fruit, fruit pod, leaves and roots of different plants belonging to multiple species. It is believed that tannins may provide plants with protection against microbial attacks.

Saponin

Saponin is glycosides with foaming characteristics in water. It is a phytochemical that can be found in most vegetables, beans and herbs. On hydrolysis, aglycones are produced.

EXPERIMENTAL

Sample Collection

The sampling was conducted in three phases (three months) to ensure representation of samples. All the results obtained are averages of the three samples collected at the three sampling sites. A total of five liquid herbal medicines/preparations meant for oral administration were purchased directly from local herbal markets in Kano state. The samples collected with their codes were as follows:

i. Bagaruwar makka (**BM**) used as remedy for typhoid.
ii Madobiya (**M**) used as blood purifier
iii. Zuwo (**ZW**) used as a remedy for diarrhea.
vi. Rai Dorai (**RD**) used for stomach pain
v. Miyar Tsanya (**MTS**) used as remedy for delivery pains.

Extraction and Fractionation Procedures

This was performed as described by Fatope *et al*., (1993) and Adoum *et al*., (1997). A portion100 gram of powdered plant material were percolated in 1 liter of absolute ethanol for two weeks, after which the extract was filtered and evaporated at 40^0C using BUCHI Rota vapor (R110). The crude extract was weighed and kept in a refrigerator.

A fraction of the crude extract was partitioned between 500 ml of chloroform and 500 ml of distilled water (ration 1:1). The mixture was shaken thoroughly and allowed to settle for 24 hours in a separating funnel. Two immiscible layers (chloroform soluble fraction and water soluble fraction) was formed, separated in glass beakers and labeled. This fraction was then concentrated using Rota vapor and stored in a refrigerator.

A portion of the chloroform soluble extract was further partitioned in a mixture of 97% methanol and petroleum ether in a ration 1:1 (300 ml: 300 ml). The methanol soluble and petroleum ether soluble fractions was again concentrated in vacuo, labeled and stored in a refrigerator.

Preliminary Phytochemical Screening

Phytochemical screenings of the ethanolic, chloroform and pet ether extracts were determined using standard methods (Harbourne, 1984).

General Alkaloid Test

One half of the ethanol solution in a test tube of the expected alkaloids was treated with normal alkaloid reagent namely. The suspected alkaloid solution was reacted with Dragendoff's reagent and reddish brown precipitates indicate the presence of alkaloids. The same alkaloid test was carried out using Mayer's reagent, Hager's reagent, Wagner's reagent, and tannic acid solution.

Fehling's Test (Standard Test for Free Reducing Sugars).

A small amount of the aqueous sample extract was reacted with a 1:1 fresh mixture of Fehling's I and II and the system heated until boiling was achieved. Boiling was allowed for 2 minutes after which a deep blue coloration is observed.

Test for Glycosides of the Saponin Type

Form Test:

A small amount of the powdered material was put in a dry test tube and moistened with 10 cm^3 of distilled water. This was filtered and shaken persistently. The filtrate was left for 30 minutes and shaken again. 2 drops of olive oil was then added and shaken again. The oil drops remain as globules.

Test for Cyanogenetic Glycosides

About 0.5 g of the powdered sample material was put in a test tube and mixed with sufficient distilled water. A moist sodium picrate paper (prepared by dipping filter paper into picric acid and then into $NaCO_3$) was suspended at the neck of the test tube and neatly corked. The arrangement was placed in an oven at 45^0 C for an hour, after which a gas was evolved from the set up, but no color change was observed on the filter paper.

Test for Cardiac Glycosides

The sample material weighing 2.0 g was boiled in 20 cm^3 of 95 % ethanol in a clean dry test tube for 5 minutes. The resulting solution was diluted with 5 cm^3 of distilled water and 3 drops of concentrated lead sub acetate solution added and mixed thoroughly. It was filtered and the filtrate divided into 2 portions for the following reaction below: -

One half of this filtrate was extracted in a separating funnel using chloroform and the chloroform layer collected in a small evaporating dish. The solvent was removed using a water bath leaving a residue of the glycosides.

Test for Tannins

The sample material (1g) was boiled with 15 cm^3 of distilled water, cooled and filtered and the filtrate subjected to the following tests: -

i. About 1cm^3 of the aqueous extract was treated with a few drops of lead sub acetate solution. The resulting solution was a cloudy whitish solution, which finally coalesced into a precipitate.

ii. The aqueous extract was diluted in the ratio 1:4 using distilled water and 3 drops of ferric chloride solution added to it and a golden yellow solution was obtained.

Test for Steroids/Terpenoids

a. Liebermann-Bustard's Test

To 0.2 g of each portion 2 cm^3 of acetic acid was added to the solution. The solution was then cooled in the ice followed by the addition of conc. Sulphuric acid carefully (Sofowora, 1993).

b. Salkowski Test

A little quantity of the extract was dissolved in 1ml chloroform, to each 1ml of conc. Sulphuric acid was added down the test tube to form 2 phases, red and yellow.

Tests for Flavonoids

Ferric Chloride Test

About 0.5 g of the extract was boiled with distilled water and then filtered. To 2 cm^3 of the filtrate, few drops of 10 % ferric chloride solutions were added to give a blue or violet color (Trease and Evans, 2002).

Procedure for Anti Microbial Screening

The anti microbial activities of the extracts from the herbal medicines were determined using some pathogenic enteric micro organisms, the microbes were obtained from the Microbiology department of the Bayero University Kano (BUK).

Preparation of Barium Sulphate Turbidity Standard

Barium sulphate standard suspension was used as turbidity standard. One percent (1% v/v) solution of sulphuric acid was prepared by adding 1 cm^3 of concentrated H_2SO_4 into 99 cm^3 of distilled water and

mixed thoroughly. One percent (1% w/v) solution of barium chloride was prepared by dissolving 0.5 g of dehydrated barium chloride in 49.5 cm^3 of distilled water. This was followed by adding 0.6 cm^3 of barium chloride to 99.4 cm3 sulphuric acid solutions and mixed thoroughly. A small volume of the turbid solution was transferred to a test tube for comparison with turbidity of test suspension contained in a similar test tube. The remaining volume of the turbidity standard was stored in a dark bottle at room temperature (Cheesebrough, 2002).

Standardization of Inoculum Density of Test Bacteria.

This was standardized by the use of overnight broth cultures by inoculating 3 loopful of well-isolated colonies of the clinical isolates in 10 ml of broth and incubated at 35^0C for 24 hours. A loopful of the overnight broth culture was diluted in 4 cm^3 of sterile physiological silane (0.8% w/v) such that its turbidity matches that of 0.5 Marc Farland (a Barium sulphate standard). This was achieved by comparing the turbidity of the test suspension with the 0.5 cm^3 McFarland turbidity standard against a background of a printed white paper (Cheesebrough, 2002).

Antimicrobial Susceptibility Test

The antimicrobial activities of the extracts and reference drugs Ciprofloxacin was determined according to the method described by Agyare *et al.,* (2012). Nutrient agar was used for the determination of the antimicrobial activities. A 10^6 cfu/ml of the test organism was used to seed nutrient agar plates. In each of these plates, wells with diameter of 4 mm were cut out using sterile cork borer and the wells were filled with different concentrations of extracts and standard antibiotic dissolved in dimethyl sulfoxide (DMSO) and allowed to diffuse at room temperature (28-30^0C) for 1 hour. The zones of growth inhibition were measured after 24 hours incubation at 37^0 C.

Determination of Minimum Inhibitory Concentration of the Extracts

Four varied extract concentrations (4000, 2000, 1000, 500 μ/ml) were prepared from the stock solution with distilled water using serial double dilution. Sterile test tubes in batches were dispensed with the extract concentrations of 10 ml of Mueller-Hinton broth respectively. Two additional test tubes of the broth containing the extract and the test organism respectively were used as controls. After overnight incubation at 35^0 C, the lowest concentration of the sample at which no turbidity was observed will be recorded as the minimum inhibitory concentration for the extract.

RESULTS

Table 1. *The result of the phytochemical screening of the petroleum ether extracts of the unregistered liquid herbal medicines.*

	MB	MC	MS	MT	STDs
Saponin	-	-	-	+	-
R/sugar	+	+	+	+	+
A/acid	-	-	-	-	-
Flavonoid	+	-	+	+	+
Tannins	+	+	+	+	+
Steroids	+	+	+	+	+
Triterpenoid	+	+	+	+	+
Glycoside	+	+	+	-	-
Digit. Glyco	-	-	-	-	-
Alkaloids	+	+	+	+	+
Cardiac/Gly	-	-	-	-	-

Table 2. *The result of the phytochemical screening of the Chloroform extracts of the unregistered liquid herbal medicines.*

	MB	MC	MS	MT	STDs
Saponin	+	+	+	+	+
R/sugar	+	+	+	+	-
A/acid	-	-	-	-	-
Flavonoid	+	+	-	+	+
Tannins	+	+	+	+	-
Steroids	+	+	-	+	+
Triterpenoid	+	+	+	+	+

Glycoside	+	+	+	-	+
Digit. Glyco	-	-	-	-	-
Alkaloids	+	+	+	+	+
Cardiac/Gly	-	-	-	-	-

Table 3. *The result of the phytochemical screening of the Ethanolic extracts of the unregistered liquid herbal medicines.*

	MB	MC	MS	MT	STDs
Saponin	+	+	+	+	+
R/sugar	+	+	+	+	+
A/acid	-	-	-	-	-
Flavonoid	+	+	+	+	+
Tannins	+	+	+	+	+
Steroids	+	+	+	+	+
Triterpenoid	+	+	+	+	+
Glycoside	+	+	+	-	+
Alkaloids	-	+	-	+	+
Cardiac/Gly	-	-	-	-	-

DISCUSSION

The results of the phytochemical screening of the petroleum ether extracts of the five unregistered herbal medicines/preparations is presented in tables 4.1, 4.2, and 4.3. Saponin was absent in all the extracts except in samples MT. saponins are poisonous bioactive constituents that are involved in plant defense system. They cause hemolysis of blood and are known to cause cattle poisoning (Barile*et al*., 2007; Kar, 2007; Ayoola *et al*., 2008). They are however found to have beneficial pharmacological use including anti-inflammatory, anti-parasitic and anti-viral properties (Just *et al*., 1998; Traore *et al*., 2000). Triterpenoid are present in all the herbal medicines. Amino acid was not present in any of the samples. Digitalis glycosides andCardiac glycosides were not present in all the samples. Herbal derived glycosides are used for the treatment of skin diseases and as anti-inflammatory, anti-microbial and anti fungal activity (Careri*et al*., 2001; Abbassey*et al*., 2007). Flavonoids, tannins, steroids and alkaloids were present in most of the tested herbal medicines.

Table4.2 showed result for the phytochemical screening of the chloroform extracts of the liquid herbal medicines. Amino acid was not found in all the samples. Flavonoids as one of the most effective bioactive compounds, was found in all the samples except in sample MS. Reducing sugar, alkaloids, tannins and Triterpenoid were present in almost all the five samples.

Tables 4.3 showed the results of the phytochemical screening of the ethanolic extracts of the unregistered herbal medicines/preparations. Alkaloids were present in samples MC, MT and STDs but were absent in samples MS and MB. Alkaloids found in medicinal preparations and herbs are widely used as anti microbial agents and are documented to have medicinal properties (Okwu, 2004; Afolabi*et al*., 2007). Flavonoids, tannins, steroids and reducing sugars were commonly found in the samples.

Table 4. *Antimicrobial activities of the petroleum, chloroform and ethanolic extracts of the unregistered liquid Herbal Medicines.*

Microorganisms Tested

Extract Tested	**Zone of inhibition (mm)**			
Conc.	***E. Coli***	***Staph***	***Shigella***	***Salmonella***
MB Pet ether 4000	15±0.10	12±0.21	13±0.15	14±0.15
2000	10±0.06	10±0.21	10±0.36	12±0.15
1000	9±0.27	8±0.27	5±0.15	10±0.12
500	0±0.00	5±0.15	0±0.00	7±0.20
Control	35±0.25	31±0.10	37±0.15	32±0.15
MC Pet ether 4000	13±0.10	12±0.32	13±0.15	15±0.11
2000	11±0.20	10±0.32	11±0.21	12±0.25
1000	9±0.10	8±0.25	10±0.22	10±0.31

500	7±0.25	6±0.20	8±0.15	7±0.15
Control	35±0.25	31±0.10	37±0.15	32±0.15
MS *pet ether* 4000	10±0.21	11±0.45	12±0.32	9±0.25
2000	8±0.27	9±0.27	9±0.32	8±0.15
1000	6±0.21	7±0.21	8±0.10	6±0.25
500	4±0.27	5±0.23	7±0.21	4±0.15
Control	35±0.25	31±0.10	37±0.15	32±0.15
MT *Pet ether* 4000	10±0.20	15±0.27	0±0.00	10±0.10
2000	8±0.15	13±0.32	0±0.00	9±0.35
1000	7±0.20	11±0.20	0±0.00	8±0.12
500	5±0.10	10±0.15	0±0.00	7±0.32
Control	35±0.25	31±0.10	37±0.15	32±0.15
STDs Pet ether 4000	14±0.15	12±0.32	9±0.36	10±0.20
2000	14±0.21	11±0.21	8±0.10	10±0.25
1000	10±0.25	9±0.21	8±0.10	8±0.15
500	9±0.21	8±0.31	7±0.06	7±0.30
Control	35±0.25	31±0.10	37±0.15	32±0.15
MB Chloroform 4000	12±0.25	9±0.20	13±0.35	12±0.20
2000	10±0.21	7±0.42	9±0.15	10±0.20
1000	9±0.15	5±0.21	7±0.36	7±0.15
500	4±0.10	0±0.00	5±0.25	4±0.10
Control	35±0.25	31±0.10	37±0.15	32±0.15
MC Chloroform 4000	15±0.15	11±0.11	16±0.38	12±0.21
2000	13±0.10	9±0.21	14±0.20	10±0.06
1000	10±0.27	7±0.20	11±0.25	8±0.21
500	7±0.21	4±0.10	9±0.31	5±0.17
Control	35±0.25	31±0.10	37±0.15	32±0.15
MS *Chloroform* 4000	0±0.00	0±0.00	10±0.15	0±0.00
2000	0±0.00	0±0.00	8±0.10	0±0.00
1000	0±0.00	0±0.00	4±0.15	0±0.00
500	0±0.00	0±0.00	0±0.00	0±0.00
Control	35±0.25	31±0.10	37±0.15	32±0.15
MT *Chloroform* 4000	12±0.21	13±0.32	8±0.15	14±0.35
2000	9±0.15	11±0.32	6±0.31	9±0.15
1000	7±0.21	9±0.32	4±0.15	8±0.31
500	5±0.06	7±0.35	0±0.00	5±0.21
Control	35±0.25	31±0.10	37±0.15	32±0.15
STDs Chloroform 4000	16±0.35	0±0.00	15±0.36	7±0.15
2000	15±0.12	0±0.00	12±0.35	0±0.00
1000	12±0.31	0±0.00	9±0.06	0±0.00
500	11±0.31	0±0.00	6±0.29	0±0.00
Control	35±0.25	31±0.10	37±0.15	32±0.15

MB Ethanolic 4000	17±0.21	13±0.49	15±0.21	14±0.27
2000	14±0.15	10±0.36	13±0.25	11±0.15
1000	10±0.10	8±0.25	11±0.30	9±0.31
500	8±0.20	5±0.27	8±0.15	7±0.32
Control	35±0.25	31±0.10	37±0.15	32±0.15
MC *Ethanolic* 4000	12±0.20	15±0.15	13±0.10	12±0.15
2000	10±0.15	13±0.36	12±0.21	10±0.31
1000	9±0.15	12±0.50	10±0.20	8±0.40
500	8±0.21	10±0.21	0±0.00	7±0.38
Control	35±0.25	31±0.10	37±0.15	32±0.15
MS *Ethanolic* 4000	13±0.15	14±0.20	10±0.31	20±0.27
2000	12±0.36	12±0.21	8±0.25	17±0.21
1000	10±0.15	9±0.27	6±0.25	15±0.25
500	7±0.27	5±0.31	4±0.29	10±0.20
Control	35±0.25	31±0.10	37±0.15	32±0.15

MT *Ethanolic* 4000	11±0.15	11±0.32	11±0.10	19±0.15
2000	10±0.32	8±0.15	9±0.06	15±0.15
1000	8±0.10	7±0.32	6±0.10	14±0.15
500	6±0.31	0±0.00	0±0.00	7±0.32
Control	35±0.25	31±0.10	37±0.15	32±0.15
STDs Ethanolic 4000	18±0.21	12±0.23	14±0.10	10±0.20
2000	16±0.06	10±0.25	12±0.21	8±0.12
1000	13±0.15	8±0.12	10±0.15	6±0.12
500	11±0.32	5±0.32	8±0.21	0±0.00
Control	35±0.25	31±0.10	37±0.15	32±0.15

DISCUSSION

The anti-microbial screening of the unregistered liquid using petroleum ether, chloroform and ethanolic extracts was investigated for antibacterial activity against *E. coli, Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* bacteria using agar well diffusion method. Standard broad-spectrum antibiotic (Ciprofloxacin) was used as the positive control and it showed values of 35±0.25, 31±0.10, 37±0.15 and 32±0.15 for *Escherichia coli, Staphylococcus aureus, Shigella* and *Salmonella typhi* respectively.

Table 4 summarized the result for the anti microbial activity of the extracts of the unregistered (liquid) herbal medicines. The activity displayed by the extracts ranged from 0.00mm – 20.00mm. For *E. coli*, the highest inhibition of 18.00mm was demonstrated by the ethanolic extract of sample STDs at a concentration of 4000 μ/ml. The highest inhibition of *staphylococcus aureus* was at 15.00mm with petroleum ether extract of sample MT and ethanolic extract of sample MC. Chloroform extract of sample MC demonstrated high inhibition of *Shigella* at 16.00mm. Ethanolic extract of sample MS demonstrated high inhibition at 20.00mm against *salmonella typhi*, and it has the highest activity among the unregistered liquid herbal medicine.

CONCLUSION

Consumption of the commonly used herbal medicine for treatment of various diseases by the people of Kano State could be supported by the findings of this work. African people rely heavily on the use and practice of traditional herbal medicines for a very long time. All this is due to the fact that herbal medicine is believed to be non-toxic, abundant, and affordable and the false belief that it has no side effect, other major reason is the lack of enough functional and conventional heath care sector.

The results of the phytochemical and antimicrobial screening of petroleum ether, chloroform and ethanolic extracts of the herbal medicines (*, MB, MC, MS, MT, STDs,)* manifests them to posses saponin, tannins, alkaloids, carbohydrate, flavonoids, glycosides, reducing sugars and Triterpenoid. Antioxidant, antifungal, anti-inflammatory activities of herbal medicines are attributed to the presence of phytochemicals.Antimicrobial screening of the selected extracts tested against four species of bacteria (*Staphylococcus aureus, Shigella, E. coli and Salmonella typhi*) showed good antimicrobial activity. Antimicrobial screening of the herbal medicines/preparations have proven the extracts to have significant inhibitory effect on most of the bacteria responsible for some of the common ailments.

REFERENCES

[1] Abbassy, M. A., Abdelgaleil, S A. M., Belal, A. S. H., Abdel, R.M. A. A., (2007). Insecticidal, antifeedant and antifungal activities of two glucosides isolated from the seeds of Simmondsiachinensis. Ind. Crop Prod.26 (3), 345-350.

[2] Abbot, N.C., Ernst, E., (1997). Patients' opinions about complementary medicine.ForschendeKomplementarmedizin; 4:164-8.

[3] Adoum, O. A., Dabo, N. T., and Fatope, M. O., (1997). Bioactivities of some Savanna plants in the Brine Shrimp lethality test and in vitro microbial Assay. International Jouirnal of Pharmacognosis: 35 (4): 1-4.

[4] Afolabi, C., Akinmoladun, E. O., Dan-Ologe, I. A., (2007). Phytochemical constituents and antioxidant properties of extracts from the leaves of Chromolaenaodorata. Sci. Res. Essays 2 (6), 191-194.

[5] Agyare, G.A., Koffuor, V.E., Boamah, F., Adu, K.B., Mensah, and L. Adu.

[6] Ayoola, G. A., Coker, H. A. B., Adesegun, S. A., Adepoju-Bello, A. A., Obaweya, K., Ezennia, E. C., Atangbayila, T.O., (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Tropical Journal of Pharmaceutical Research 7 (3), 1019-1024.

[7] Barile, E., Bananomi, G., Antignani, V., Zolfagari, B., EbrahimSajjadi, S., Scala, F., Lanzotti, V., (2007). Phytochemical screening and antimicrobial assessment of Abutilon mauritianum, bacopsmonifera and Daturastramonium.Phytochemistry 68, 596-603.

[8] Barnes, J., (2003). Quality, efficacy and safety of complementary medicines: fashions, fact and the future. Part 1. Regulation and Quality. Br J Clin Pharmacol; 55(3): 226-33.

[9] Careri, M., Elviri, L., Mangia, A., (2000). Liquid-chromatography-UV determination and liquid chromatography, atmospheric pressure, chemical ionization, mass spectrometric and characterization of sitoterol and stimasterol in soy bean oil. J. Chromatogr. 935, 249-257.

[10] Cheesebrough, M., (2002). District Laboratory Practice for Tropical Countries parts two. Cambridge University Press. UK. P 62-69.

[11] Ernst, E. White, A.: The BBC survey of complementary medicine used in the UK. ComplTher Med 2000, 8:32-6.

[12] Fatope, M. O., Ibrahim, H. and Takeda, Y., (1993). Screening of higher plants reputed as pesticides using the brine shrimp lethality assay. International Journal of Pharmacognosis 31 (4): 250-254.

[13] Harborne JB, (1984). Phytochemical methods, 2nd edition, Chapman and Hall publications, London, NewYork, pp. 288.

[14] Huxtable R.J. The harmful potential of herbal and other plant products. Drug Saf 1990; 5:126-36 (Suppl.).

[15] Just, M. J., Recio, M.C., Giner, R. M., Cuellar, m. j., Manez, S., Bilia, A. R., Rios, J. L., (1998).Anti-inflammatory activity of unusual lupine Saponin from Bupleurumfruticescens. Planta Med. 64 (5), 404-407.

[16] Kar, A., (2007).Pharmacognosis and pharmacobiotechnology. Second ed. New age int. Ltd. New Delhi. Pp. 332-600.

[17] Okwu, D. E., (2004).Phytochemical and vitamin content of indigenous spices of southeastern Nigeria. J. Ethnopharmacol. 137(1), 523-533.

[18] Sofowora, E. (1993). Medicinal Plants and Traditional Medicines in Africa 1st ed.. New York, John Wiley and Sons. Pp.1-23.

[19] Traore, F., Faure, R., Ollivier, E., Gasquet, M., Azas, N., Debrauwer, L., Keita, A., Timon-David, P., Balansard, G., (2000).Structure and antiprotozoal activity of Triterpenoid saponins from Glinusoppsitifolius. Planta Med. 66 (4), 368-371.

[20] Trease, E.G., Evans, C.W., (1972). Pharmacognosy. 10thed, BailliereTinali- London. Pp. 332-333.