PHYTOCHEMICAL SCREENING OF SOME PLANT PREPARATIONS HAVING POTENTIAL FOR THE TREATMENT OF TYPHOID FEVER

UMMU RABI'U R.A[1], HARUNA SA'IDU[2], FARUK UMAR[3], MUSA MUNTARI[1], SANI ALIYU IBRAHIM[1]

[1]Department of Microbiology, Faculty of Science, Gombe State University
[2]Department of Biological Sciences, Faculty of Sciences, Gombe State University
[3]Department of Biological Sciences, Abubakar Tafawa Balewa University Bauchi

ABSTRACT

Study of the microbiological quality of selected herbal preparation used in the treatment of typhoid fever sold in Gombe metropolis, Gombe State was conducted with a view to determine their anti-typhoid activity. Five (5) herbal preparations were selected and tested namely; Med-bunch, Al-muwafaqa, Mau'shifa, Yellow cassia, and Addawau'l humma and compare with the standard drug Ciprofloxacin which is commonly used for the treatment of typhoid. Disc diffusion method was used for the determination of antibacterial activity of the herbs. Results of phytochemical screening revealed that all the preparations contain most of the secondary metabolites namely Alkaloid, Flavonoid, Tannins, Glycosides, Anthraquinone, Saponins and Steroid). Med-bunch has the highest quantity of alkaloids followed by Al-muwwafaqa and Yellow cassia. Result of antibacterial test reveal that Addawa'ul humma exhibit the highest anti *Salmonella* activity at a concentration of 100%, followed by Yellow cassia extract. Addawa'ul humma has the highest concentration of phosphate and sulphate of 0.72 mg/kg and 0.2 mg/kg respectively while Al-mufaqa has the least amount 0.09 mg/kg and 1.09 mg/kg). Result of this study advocate for further validation of the potential use of these preparations in the treatment of typhoid giving more priority to dosage and purity.

Keywords: Typhoid fever, herbs, metabolites, antibacterial, phytochemicals analysis

INTRODUCTION

Antibiotics have long been considered as the major main basis for the therapy of bacterial and fungal infection (Harbottle *et al*., 2006). This resulted into the Bacterial developing resistance asa results of inappropriate use of antibiotics (Uniyal *et al*., 2006). Research has indicated infection due to bacterial action are responsible for many deaths in each year (Betoni *et al*., 2006), and Currently used antimicrobials are failing to bring an end to many bacterial infections due to super resistant strains. For this reason, researches are currently ongoing for the bioprospecting for natural anti-microbial substances from plant (Lewis and Ausubel, 2006). Herbal medicine was a very ancient art of healing one that use both perceptual and intellectual awareness as well as focus on outcome and antibacterial activity also reported (Bonjar, 2004).

Despite the available studies on the antimicrobial potential of different medicinal plants (Nassiri-Asl et al., 2007), Novel phytochemical substance of plant origin has been shown to inhibit the growth of bacteria (Varsha *et al*., 2013; Lewis and

Ausubel, 2006)). This is because, bioactive compound in the plants resulted in the discovery of new medicinal substance (Ates and Erdourul, 2003). For instance, traditionally used medicine from *Glycyrrhiza glabra* and *Azadadirachta indica* having antibacterial activity was also reported (Varsha *et al*., 2013). *Glycyrrhiza glabra* belonging to family *Papilionaceae* contain compounds like Saponin, glycoside that is glycyrrhizin, Flavonoids and many other constituents (Usmanghani, 1997).

Herbal preparations have wide range of uses including treatment of many diseases such as malaria, jaundice, menstrual pain, waist pain, piles, delay in ejaculation, hypertension, rheumatism, and many others in Nigeria. Herbal preparations are not only used in Nigeria but also in developed countries such as UK, China, United States, India, for the treatment of mood disorders, particularly depression; for relief of anxiety and stress, insomnia; treatment of urinary tract infection, decrease kidney stone; to lower cholesterol levels and blood pleasure, as immune stimulants that help increase resistant to cold, relief from migraine headache and arthritis, healing of wounds; burns; skin ulcers; heart failure; hypertension (Ate and Evdowul, 2003; Brindha *et al*., 2012).

In Nigeria, there is proliferation of herbal products on the market, not much has been done in this field. The producers of the herbal preparations, in Nigeria do not have the required laboratories or expertise to perform quality control on the preparations they produce. This brings the problem of inconsistency on the quality of the herbal preparations in the country. This research work, therefore, assesses the phytochemical properties of herbal preparations on the Nigerian market using harmonized procedures.

MATERIAL AND METHODS

Composition of the herbal preparation

The composition of Medbunch are *Cleistopholis patens, Xlopia aethiopica, Srcuridaca Longedunculata, Mandifera Indica, Morinda Lucida, Zanthoxyylum Zanthoxylloides, Zingiber officinales,* for Addawa'ul humma (teaming) are treated water, guava leaves, mango leaves, neem tree leaves, bitter orange tree leaves and umbrella tree leaves, for Muwafaqa are *Ficus gnaphalocarpa, Mandifera indica, Cidium guajava, and Kuma ficus.* In the case of Ma'u shifa its composition are basically *Mandifera indica, Morinda lucida,* Mango leaves, Neem tree leaves and Umbrella tree leaves. The entire experimental preparation was done in Gombe, Gombe State which is a city in north eastern Nigeria.

Determination of Physicochemical Parameters of Herbal Preparation

pH: The pH of the herbal preparations was determined using pH meter (model: HANNA instrument model YSI 63). The electrode was standardized using a buffer solution according to Nuhu, (2015). The electrode of the pH meter was dipped into the water sample for 2-3 minutes and measurement was recorded.

Nitrate: Nitrate was measured using a testab water investigation kit as recommended by Nuhu, (2015). A test tube (0106) was filled with water to 5ml line. One Nitrate testab 1 (2799A) was added and the test tube was capped and mixed until the tablet has

disintegrated fully. One tablet of Nitrate CTA testab 2 (NN-3703A) was added again and immediately the test tube was capped and mixed for 2 minutes in order for the tablet to disintegrate. It was then allowed to settle for 5 minutes. The colour of the sample was compared with the Nitrate colour chart (5891-CC). The result was recorded as milligram per litre (mg/l) Nitrate

Alkalinity: Alkalinity determined using a testab water investigation kit as recommended by Nuhu, (2015). A test tube (0788) was filled with herbs to 100ml line, followed by the addition of Alkaline testab (3920A) and colour of the solution changes from green to pink. This was then matched with the alkaline colour chart (5893-CC). Total alkalinity will be determined by multiplying the number of tablets by 40.

Phosphate: A test tube (0106) was filled with herbs sample to 5ml line. One Phosphate testab (5422A) was added. The test tube was capped and mixed until the tablet disintegrated fully. The solution could stay for 5 minutes. The colour of the sample was compared with the Phosphate colour chart (5892-CC). The result will be recorded as milligram per litre (mg/l) Phosphate

Phytochemical Screening

Each of the herbal drugs selected were analyzed for its phytochemical constituent without any extraction with relevant solvent.

Test for flavonoids: A 5 ml of the herbal drug were added to a concentrated Sulphuric acid (1 ml) and 0.5 g of Mg. A pink or red coloration that disappear on standing (3 min) indicated the presence of flavonoids.

Test for tannins: Two methods were used to test for tannins. First, about 1 ml of the drug was added in 2 ml of water in a test tube, 2 to 3 drops of diluted ferric chloride solution were added and observed for green to blue-green (cathechictannins) or blue-black (garlic tannins) coloration. Second, 2 ml of the aqueous extract was added to 2ml of water, a 1 to 2 drops of diluted ferric chloride solution was added. A dark green or blue green coloration indicates the presence of tannins.

Test for saponins: To 1 ml of aqueous extract were added few volumes of distilled water in a test tube. The solution was then be shaken vigorously and observed for a stable persistent froth for 20 min.

Test for alkaloids: Three methods were used to test for alkaloids; (i) A 10ml of the extract was evaporated under water bath to obtain the dry residue, followed by the addition of 1.5 ml HCl (2 %) acid solution. After that, 1 to 2 drops of Mayer's reagent and Wagner was added, a yellow- white precipitate indicates the presence of the alkaloidal base. (ii) A 10 ml of the extract was evaporated under water bath to obtain the dry residue; it will then be dissolved in 5 ml of HCl (2 N) and filtered. A few drops of Mayer's reagent and Wagner's were added; the presence of precipitate indicates the alkaloids. (iii) A 15 ml of the aqueous extract was added 2 ml of NH_4OH a 10 % (pH =7). The alkaloid was extracted 3 times with 10 ml chloroform. The chloroform layer was then washed 3 times with 2 ml of HCL (10 %). These were divided into two portions. Mayer's reagent was added to one portion and Wagner's reagent to the other. The formation of a brown or white

precipitate was regarded as positive for the presence of alkaloids.

Test for anthraquinone: Eight (8) ml of the extract was treated with the Bornträger reagent, a positive test is revealed on the appearance of a bright color change from orange red to purple.

Test for sterols and steroids: Sterols and steroids were sought by the reaction of Liebermann. Ten (10 ml) ml of extract will be evaporated. The residue was dissolved in 0.5 ml of hot acetic anhydride follow by the addition of 0.5 ml of the filtrate chloroform. The mixture was then treated with the Liebermann-Burchard. The appearance, at the interphase, a ring of blue-green, showed a positive reaction.

Microbiological Assessment of the Herbal Preparation

Herbal preparations collected were tested for the bacterial and fungal load by pour plate method (Olaleye, 2007). All the microbial contaminants were characterized at least to genera level (Cheesbrough, 2016). Five (5) herbal products were selected for this study after obtaining good literature and activity.

RESULTS

Phytochemical Characteristics of Herbal Preparations

The screening for phytochemical components of herbal preparations conducted in this research was shown in Table 1. All the herbal preparations contain phytochemical such as alkaloid, anthraquinone, flavonoid, glycosides, saponins and steroids at different quantity.

Table 1: Phytochemical Screening of herbal preparations of the plant sample

Phytochemicals	**Herbal preparations and Test**				
	Med Bunch	**Al-Muwafaqa**	**Ma'u-shifa**	***Y. Cassia***	***A. Humma***
Alkaloids	+++	+	+	++	+
Anthraquinone	++	+	++	+	+
Flavonoid	+	+	+	++	++
Glycosides	+	+	+	++	+
Saponins	+	+	+	++	+
Steroids	++	+	+	++	+
Tannins	+	+	+	+	++

Key: +++ = Present in high amount, ++ = Present in moderate amount, + = Present in small amount, - = Absent

Physicochemical Properties of the Herbal Samples

The assessments of physicochemical properties of the herbal preparations were presented in Table 2. The results showed that Ma'ushifa and yellow cassia samples is acidic, while med bunch, Al'mawafaqa and Addawa'ul Humma are alkaline, with some

quantity of phosphate of about 1.02-4.20mg/kg.

Addawa'ul humma has the highest sulphate content of 0.72 mg/kg, followed by Yellow Cassia with 0.42 mg/kg and Ma'u shifawith 0.23, medbunch 0.11 and the least is Al-muwafaqa with 0.09 mg/kg. In the case of phosphate, Addawa'ul Humma has the highest of 4.2mg/kg while the Almuwafaqa was recorded as the herbal preparation with the least phosphate content of 1.09mg/kg. Relatively, equal pH value was received for Med-bunch, Almuwafaqa and Addawa'ul humma (Table 2).

Table 2: Physicochemical properties of the herbal samples used in this study

Herbal samples	Physicochemical Properties				
	Sulphate mg/Kg	Phosphate mg/Kg	Acidity (mg/L)	Alkalinity (mg/L)	pH
Med-Bunch	0.11	2.40	0.63	0.82	7.31
Al-Muwafaqa	0.09	1.09	0.41	0.88	7.49
Ma'u Shifa	0.23	1.32	0.92	0.09	6.92
Yellow cassia	0.42	3.1	0.98	0.04	6.03
Addawa'ul Humma	0.72	4.2	0.51	0.92	7.52

Microbiological Quality of the Herbal Preparations

The assessment of microbiological quality of the herbal preparations was compile and presented in Table 3. The results revealed that only Ma'u Shifa recorded a total colony count of 2 ± 1 and no faecal coliforms were detected in the other herbal samples. *E. coli* was used here as a model example inorder to determine the quality of these preparations before subjecting them on *Salmonella typhi.*

Antibacterial efficacy of the Herbal Samples on the Salmonella isolates

Antibacterial activity of the Herbal Samples the Salmonella isolates from this study shows a highest zone of inhibition with respect to highest concentration (100%) across all the herbal samples (Table 4). Addawa'ul humma has the highest zone of inhibition of 22.4 than Med-bunch herbal sample with 11.2. But when compared with control (ciproflaxacine) its zone of inhibition was found to the highest of all zone of inhibition of 28.1.

Table 3: Microbiological quality of herbal preparations of the plant sample

Herbal Samples	Total coliform count	*E. coli* count
Med Bunch	0	0
Al-Muwafaqa	0	0
Ma'u-shifa	2 ± 1	0
Y. Cassia	0	0
A. Humma	0	0

Table 4: Anti-salmonella Activity of the Herbal Samples on the Salmonella isolates from this study

Phytochemicals	Concentrations (%) and Susceptibility/Zones of Inhibition (mm)				
	20	**40**	**60**	**80**	**100**
Med-Bunch	0	0	5.1 ± 2.1	6.8 ± 4.0	11.2 ± 2.3
Al-Muwafaqa	5.1 ± 1.3	7.9 ± 1.4	8.2 ± 1.2	8.8 ± 1.9	9.4 ± 2.1
Ma'u Shifa	0	0	0	7.7 ± 1.4	9.2 ± 1.1
Yellow cassia	0	5.2 ± 1.7	9.5 ± 1.0	12.9 ± 1.1	13.4± 2.1
Addawa'ul,humma	8.0 ± 1.3	11.2 ± 1.0	13.4 ± 2	18.1 ± 1.31	22.4 ± 1.1
Ciproflaxacin	0	0	0	0	28.1 ± 2.4

Values are expressed as Means ± Mean Deviation

DISCUSSION

The phytochemical compounds found were present in small to moderate amounts in all the samples tested, with yellow cassia as the highest, followed by Med Bunch, where alkaloids were found in high amount. All the herbal preparations use in this report for the anti-typhoidal, were found to contain the most important secondary metabolite such as alkaloid and flavonoids but med-bunch happens to contain more alkaloid than the rest of the herbal samples. Yellow cassia and Addawa'ul-humma contain more flavonoids. Such components were reported exhibit their antimicrobial effects by destroying the cell wall of antigen and hence render it death (Tsou *et al.*, 2016; Olaleye, 2007). Steroid was also found in enough quantity in all the five-herbal preparation. Except for Ma'u-shifa and Addawa'ul-humma, for the three trials carry out for the phytochemicals only Al-Muwafaqa happens to contain no Anthraquinones. Research has shown that more than 65 % of Nigerians in the rural areas rely heavily on locally made herbal products (WHO, 2008). In this study, only Ma'u Shifa recorded a total colony count of 2 ± 1 and no faecal coliforms was found in the samples, which indicated a very low level of contamination (Table 2). Although there are research studies showing that certain plant species have phytochemical constituent, for instance Somia and his co-workers (2016) tested methanol extracts of *Glycyrrhiza glabra* and *Azadirachta indica* and have profound antimicrobial activity against both gram positive and gram negative bacteria due to the presence of several phytochemical. In this research we proposed novel herbal preparations that have phytochemicals comparatively higher than those obtained from individual plant species giving them the ability to be used for the treatment of bacterial infection that causes typhoid fever.

Furthermore, these preparations were formed using available basic raw materials describe in the earlier section of the paper. Result of this experiment further reported that Addawa'ul humma was found to be the effective preparation against *Salmonella typhi* which is due to its ability to produce the highest zone of inhibition apart from control which is ciprofloxacin. The order of increasing effectiveness of the other preparations follows the trend as follows, Addawa'ul humma>Yello cassia>Muwa'u shifa>Al-muwafaqa>Med-bunch.

The potential of antibacterial effect of preparations different concentrations were tested against *Salmonella typhi*. The justification of varying the concentrations of the plant extract is due to the following reason; (1) to determine the optimum level of the preparation to inhibit the growth of the bacteria, (2) to determine the tolerance level of the bacteria against the increase in the concentrations of the preparation. The results presented in Table 4 showed that at no zone of inhibition produced at 20 and 40% (v/v) concentration for Med-bunch, but at 60, 80 and 100 %, the zone of inhibition was observed to be 5.1, 6.8 and 11.2mm respectively. Similarly, Almuwafaqa produced zone of inhibition between 5.1-9.4 mm under same concentrations.

For Addawa ulhumma, the ranges of inhibition zone were 8.0-22.4mm. It was however noted that when compared with control, highest zone of inhibition was recorded of 28.1 mm. This confirmed that as the concentration of the preparations increases, its activity of against the bacteria also increases and vise vasa (Table 4). This might be due to the presence of phytochemical such as cardiac glycosides, flavonoids, alkaloid, Carbohydrate, tannins, terpens, and saponins which were all confirmed in this research and were presented in Table 4 of this study. This result was found to agree with the research of Djeussi *et al*., (2013) which found same secondary metabolite of the plant extract when screen out the extract obtained from other plant such as *A. digitata* leaves, *A. mannii* leaves, and *A. Alboviolaceum* fruit, *A. Polyanthum* fruit and *O. Gratissimum* twigs. However, when Anibijuwon *et al*., (2012) tested the effect of *Vernomia amygdalina* on *Klebsella pneumonea*, he reported that no effect of the plant extract was observed on the bacteria. Perhaps the reason why this study suggested positive result was due to the differences in the combination of phytochemical composition found in the phytochemicals. Therefore, this research was the first to report the potential effect of this locally made preparation against the activity of *Salmonella typhi* giving it the ability to be used for the treatment of typhoid fever that is resistance to synthetic drug.

CONCLUSION

It is now obvious that exclusive reliance on one particular health practice cannot assuage the health needs of the populace. The present study indicated that the different herbal preparations have potential for the treatment of typhoid fevers which due to the presence of alkaloid, tannin, flavonoid, anthraquinone, saponin and steroid. In terms of quality, Ma'u shifa is the only preparation with highest bacterial load of 2. Addawa'ul,humma was observed to be the most effect preparation against the activity of typhoid fever.

REFERENCES

Ates, D.A., Erdourul, Z.T. (2003). Antimicrobial activities of various medicinal and commercial plant extracts. *Turkish Journal Biology*. 27, 157- 162.

nibijuwon, I.I., Oladepo, B. O., Adetitun, D. O., Kolawale, O. M. (2012). Antimicrobial Activities of *Vernonia amygdalina* against Oral microbes. *Journal of pharmacology*. 6(3): 178-185.26.

Betoni, J.E., Mantovani, R.P., Barbosa, L.N., Di, Stasi, L.C., Fernandes,

J.A. (2006). Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. *MemInstOswaldo Cruz*. 101, 387-390.

Bonjar, S. (2004). Evaluation of antibacterial properties of some medicinal plants used in Iran. *Journal of Ethnopharmacological*. 94, 301-305.

Brindha, M.S., Kariyarasi, S., Annadurai, N.S., Gangwar, S.K. (2012). Antimicrobial activity in leaf extract of Neem (*Azadirachta indica*). *International journal of science and Natural*. 3, 110.

Djeussi, D. E., Noumedem, J. A., Seukep, J. A., Fankam, A. G., Voukeng, I. K., Tankeo, S. B., Kuete, V. (2013). Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. *BMC complementary and alternative medicine*, *13*(1), 164.

Harbottle, H., Thakur, S., Zhao, S., and White, D.G. (2006). Genetics of antimicrobial resistance. *Animal Biotechnology*. 17, 111-124.

Lewis, K., and Ausubel, F.M. (2006). Prospects for plant-derived antibacterials. *Natural Biotechnology*. 24, 1504-1507.

Nassiri-Asl, M., Saroukhani, S., Zamansoltani, F. (2007). Anticonvulsant effects of aqueous extract of *Glycyrrhiza glabra* root in PTZ-induced seizure in mice. *International Journal of Pharmacology*. 3, 432–434.

Olaleye, M.T. (2007). Cytotoxicity and antibacterial activity of methanolic extract of Hibiscus sabdariffa. *Journal of Medicinal Plants Research.* 1, 009–013.

Somia, Gul., Asma, E., and Zehra, A. (2019). *Glycyrrhiza glabra* and *Azadirachta indica* against *Salmonella Typhi*: Herbal Treatment as an Alternative Therapy for Typhoid Fever. *Archives of medicine*. 2(1), 1-3 (58).

Tsou, L. K., Lara-Tejero, M., RoseFigura, J., Zhang, Z. J., Wang, Y. C., Yount, J. S., Lam, W. (2016). Antibacterial flavonoids from medicinal plants covalently inactivate type III protein secretion substrates. *Journal of the American Chemical Society*, *138*(7), 2209-2218.

Uniyal, S.K., Singh, K.N., Jamwal, P., and Lal, B. (2006). Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. *Journal Ethno biology and Ethnomedicine*. 2, 14.

Usmanghani, K. (1997). Indyusenic medicine, Traditional medicine of Herbal, Animal and mineral origion in Pakistan.

Varsha, S., Agarwal, R.C., PandeySonam, N.D. (2013). Phytochemical Screening and determination of Anti-bacterial and anti-oxidant potential of *Glycyrrhiza glabra* root extract.