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Phytochemical and Antibacterial Profile of Some Liquid Herbal Preparations Sold in Abia State, South-Eastern Nigeria

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Abstract

The use of liquid herbal preparations (LHPs) in the treatment and management of human diseases has long been practiced before the advent of chemotherapy. The beneficial medicinal effects of liquid herbal preparations materials typically result from the secondary products present in the liquid herbal preparations although, it is usually not attributed to a single compound but a combination of the metabolites. This study was carried out to evaluate the antibacterial and phytochemical profile of some liquid herbal preparations in selected markets of Abia State using biochemical and microbiological assay. Preliminary qualitative phytochemical analysis of the liquid herbal preparations inferred the presence of alkaloids (30%), quinones (6%), saponins (7%), flavonoids (19%), glycosides (17%) and tannins (22%). The antibacterial activities of 20 LHPs with bioactive properties against 3 tested clinical bacterial isolates; Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa, from hospital samples was evaluated using agar well diffusion methods. Inhibitory zone diameter (IZD) had range of 6-20mm for all bacterial isolates. The pH values of 20 bioactive LHPs range from 3.75-6.76. In conclusion the study revealed consistent presence and varied occurrences of different phytochemicals and antibiotic properties in various herbal preparations which may be taken to indicate that the products are effective. It is also essential to investigate all LHPs to be used as alternative medication in humans, to ensure that the concentrations used will definitely cause inhibition of the specific bacterial strains and meet its therapeutic claims.

***Key words**: Liquid Herbal Preparations; Phytochemical; Antibacterial Activity.*

INTRODUCTION

Consumption of herbal medicinal products is increasingly becoming popular, often perceived as being natural (Soforawa, 1993; Adeleye *et al.*, 2005). Herbal medicines are prepared from a variety of plant materials which are potent biological agents. These plant raw materials harvested at various stages of development contain active constituents that may also be at different stages of biosynthesis forming potent phytochemicals (Faleye *et al.*, 2012).

Phytochemicals are chemical constituents formed in the plant's normal metabolic processes often referred to as "secondary metabolites" of which there are several classes including alkaloids, anthraquinones, coumarins, fats, flavonoids, glycosides, gums, iridoids, mucilages, phenols, phytoestrogens, tannins, terpenes, terpenoids, ascorbic acid, folic acid and vitamin E, to mention a few (Bruneton, 1999; Falodun *et al.*, 2006,). Many phytochemicals have been shown to be bioactive, showing exhibit pronounced biological activity in other living organisms (Harborne, 1973). Alkaloids have been reported to posses antibacterial properties with pharmacological effects which could be associated with the inhibition of nucleic acid, protein and membrane phospholipids biosynthesis and intercalate with DNA (Tanaka *et al.*, 2006). The compound has protective role in animal and it is used in medicine especially the steroidal alkaloids which constitutes most of the valuable drugs (Atta-ur-Rahman *et al.*, 1995).

Studies have shown that saponins have various biochemical properties which are therapeutics. The occurrence of steroidal saponins from numerous studies showed their importance and interest in pharmacy due to relationship with such compounds as sex hormones (Edeoga *et al.*, 2005). Additionally, saponins are equally used in medicine and pharmaceutical industries because it's used in the preparation of insecticides, various drugs and synthesis of steroid hormones (Okwu, 2008). Studies have reported that

flavonoids being phenollic compounds are water which are capable of preventing oxidative cell damage and have strong anticancer activity (Okwu, 2008). Many diseases are known to be exacerbated by the presence of free radicals such as superoxide and hydroxyl and flavonoids have the ability to scavenge and effectively mop up these damaging oxidizing species (Kaufman *et al.*, 1999).

Studies have reported that tannins have been found to form irreversible complexes with proline rich proteins resulting in the inhibition of the cell protein synthesis. Tannins bind proteins and adhesions; inhibit enzymes and complex with cell wall (Iqbal *et al.*, 2006). All these explain the therapeutic activities of tannins. It is therefore evident that Liquid Herbal Preparations with certain phytochemicals have therapeutic importance and maybe were responsible for the antibacterial activities exhibited by some LHPs. The oxidation inhibiting activity of tannins have been known for a long time and it is assumed to be due to the presence of garlic and diagollic acids, with styptic and stringer properties of tannic acid which was used in the treatment of inflammatory skin eruption and bowel conditions (Haslam,1996). Their consistent presence in the herbal products in the present study may be taken to indicate that the products are effective. Their varied occurrences in various herbal preparations will however indicate that probably, their therapeutic effect(s) are not the direct effect of a single group or compound, but rather that the compounds possibly act in combination to bring about an effect.

A number of reports have described the antibacterial potency of herbal remedies and indicated that the apparent effectiveness of plant antimicrobials is largely due to its activity against the permeability barrier of bacterial cell wall, and thus the activity of the majority of plant antimicrobials is greater against Gram-positive bacteria, which lack this barrier (Nostro, *et al.*, 2000; Wallace, 2004).

The combined actions of these substances tend to increase the activity of the main medicinal constituents by speeding up or slowing down its assimilation in the body. The main differences between whole herbs and traditional extracts on one hand, versus individual vitamins, minerals, and isolated phytochemicals, is the principle of synergy, which is the complex interactions among the many constituents of herbs give rise to its unique characteristics and healing properties (Robbers *et al.*, 1999; Falodun *et al.*, 2006). Phytochemicals present in active herbal medicinal products like tannins and flavonoids are thought to be responsible for anti-diarrhoeal activity by increasing colonic water and electrolyte reabsorption. Others act by soluble, antioxidants and free radical scavengers inhibiting intestinal motility (Bankole *et al.*, 2007). Some of the active ingredients are potentially toxic, while others, though not yet classified as nutrients, their substances are known for the prevention and treatment of health conditions, including cancer, heart disease, diabetes, and high blood pressure (Palombo, 2006). The synergistic effects of organic compounds with antimicrobial activity in association with its phytochemicals can provide effective therapy against pathogenic microorganisms.

This study was carried out to evaluate qualitatively the phytochemical components and antibacterial activities of some herbal preparations in selected towns in Abia State, using harmonized biochemical and microbiological assay.

MATERIALS AND METHODS

Study area and sample collection

A total of 150 samples were purchased from selected towns in Abia State (50 each from Umuahia, Ohafia and Aba). All the samples collected from the sites were analyzed in Postgraduate Microbiology/ Biochemistry Laboratory, all in the College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State. These herbal preparations which had various therapeutic claims were purchased from different vendors to get as many different brands as possible. The samples were within their shelf lives, were kept at room temperature and were analyzed within two weeks of collection.The sealed bottles of the herbal preparations were cleaned with 70% ethanol before opening to prevent contamination.

The herbal preparations used for this study were all in liquid formulations with different plant parts (i.e. bark, roots, wood etc) which are either active ingredients or preservatives. All herbal preparations were given codes - LHP and numbers for specificity.

The shelf life, phyto-composition, diluents used in the preparation of liquid herbal preparations (LHPs) were collated through questionnaire and interactions with herbal vendors. Hydrogen ion concentration (pH) characteristics of LHPs collected were assessed using a PHS-25 Ph/conductivity meter.

Qualitative phytochemical assay were carried out for all liquid herbal products using standard methods. A portion of each herbal product was subjected to biological screening for the identification of the major secondary metabolites (Obadoni and Ochuko, 2001; Palombo, 2006; Anowi *et al.*, 2012; Barbosa *et al.*, 2012).

Phytochemicals tested for includes; glycosides, tannins, saponins, flavonoids, alkaloids and quinines

Test for Glycosides: One millilitre of herbal samples was added to 1mL of water and then aqueous Sodium hydroxide (NaOH) solution was added. Observation of yellow colouration indicates the presence of glycosides (Wagner and Bladt, 2009).

Test for Tannins: Two millilitres of herbal samples was added to 1% gelatin solution containing sodium chloride. Observation of white precipitate shows the presence of tannins (Wagner and Bladt, 2009).

Test for Saponins: Two millilitres of different liquid herbal samples was shaken well with 5mL of distilled water and then heated to boil. Observation of frothing evolution shows the presence of saponins (Wagner and Bladt, 2009).

Test for Flavonoids: Two millilitres of different liquid herbal samples was treated with few drops of sodium hydroxide solution. Observation of intense yellow colouration which becomes colourless on addition of dilute acid shows the presence of flavonoids (Wagner and Bladt, 2009).

Test for Alkaloids: One millilitre of different liquid herbal samples was dissolved individually in dilute Hydrochloric acid, filtered and treated with Wagner's reagent (Iodine in Potassium Iodide). Observation of brown/reddish precipitate shows the presence of alkaloids (Wagner and Bladt, 2009).

Test for Quinones: One millilitre of different liquid herbal samples was dissolved individually in 2mL dilute sodium hydroxide. Observation of blue green/red precipitate shows the presence of quinines (Wagner and Bladt, 2009).

Inhibition Zone Diameter Measurement (Agar Well Diffusion Method)

Three clinical bacterial isolates (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) from wound infections were obtained from the Microbiology Laboratory, Federal Medical Centre, Umuahia, Abia State, Nigeria; for the antibacterial screening of the liquid herbal products. Some morphologically similar colonies of these clinical isolates were collected with a sterile wire loop and transferred for growth in peptone broth and incubated at 37°C. The visible turbidity was equal to the 0.5 McFarland standard prepared by adding 0.05 millilitres of barium chloride to 9.95 millilitres of sulfuric acid (Hugo and Russel, 2004 and CLSI, 2013). Intermediate dilutions of LHPs were prepared by making successive 1:2, 1:4, and 1:8 dilutions or by making serial twofold dilutions. One part of these dilutions was added to nine parts of molten Mueller-Hinton agar (MHA) (Oxoid, UK) and allowed to equilibrate in a water bath to 45 to 50°C. The molten Mueller-Hinton agar and dilutions of LHPs was mixed thoroughly and poured aseptically into Petri dishes on a level surface to result in an agar depth of 3 to 4mm. Mueller-Hinton agar plates without LHPs was used as negative controls. Six wells (6mm) were made in the agar with aid of cork borer No. 4. The wells were sealed at the bottom with molten sterilized agar, Aliquot of each well-mixed adjusted and diluted bacterial suspensions (10^7 CFU/mL) was placed into the corresponding wells with standardized pipettes. A growth-control plate (no LHPs) was inoculated and a second growth control plate to ensure there was no contamination or significant antimicrobial car+ryover during the inoculation. Antibiotic disc (Ciprofloxacin- 5µg) used as control was placed on the agar aseptically. The plates were then incubated at 37°C for 24 hours. The zone diameters of inhibition produced by each dilutions of the liquid herbal product and that of the antibiotic disc was measured and recorded in millimetres (CLSI, 2013).

RESULTS

Table 1 show the distribution and frequency of phytochemicals identified qualitatively in liquid herbal preparations (LHPs). Six phytochemicals namely, alkaloids, quinones, saponins, flavonoids, glycosides and tannins occurred in different proportions. Alkaloids occurred most, being detected in 47(30%), saponins in 11(7%), flavonoids in 30(19%), glycosides in 27(17%) and tannins in 34(22%) of 150 LHPs. Quinones were only identified in only 9 (6%) LHPs. Presence of these phytochemicals in these LHPs, depends on numerous factors which include plant part used, diluents used for extracts, shelf life and preparation process.

Table 2 shows shelf life, pH, composition, diluents, therapeutic characteristics and antibacterial activities (inhibitory zone diameter) in mm of 20 liquid herbal preparations. Shelf life of 20 bioactive LHPs range from 1-8 weeks, pH values of range from 3.75-6.76. The phyto-composition most being plant parts of each LHPs varies with it therapeutic claims and diluents.

Table 3 shows the details of the mean inhibitory zone diameter (mm) of some liquid herbal preparations and standard inhibitory zone diameter (mm) of conventional antibiotics against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Mean IZD of LHPs against *S. aureus*, *E. coli* and *P. aeruginosa* was significant at $P<0.05$ to standard IZD of some conventional antibiotics. *Escherichia coli* were more susceptible to most LHPs.

Table 1: Distribution and Frequency of Phytochemical in Some Liquid Herbal Preparations in Abia State

		Number and (%) Proportion			
S/N	**PHYTOCHEMICALS**	**Umuahia**	**Ohafia**	**Aba**	**Total**
1	Alkaloids	15(29)	21(37)	11(22)	47(30)
2	Quinones	2(4)	4(7)	3(6)	9(6)
3	Saponins	3(6)	3(5)	5(10)	11(7)
4	Flavonoids	11(22)	6(11)	13(26)	30(19)
5	Glycosides	8(16)	11(19)	8(16)	27(17)
6	Tanins	12(24)	12(21)	10(20)	34(22)
	TOTAL (%)	**51(100)**	**57(100)**	**50(100)**	**158(100)**

Table 2: Shelf Life, Physico-Chemical, Composition, Therapeutic Characteristics and Antibacterial Activities (Inhibitory Zone Diameter) in mm of Some Liquid Herbal Preparations

S/N	SAMPLE CODE (LHP)	SHELF LIFE (WEEKS)	pH	INGREDIENTS CLAIM	THERAPEUTIC CLAIM	IZD (mm) *Staphylococcus Aureus*	*Escherichia Coli*	*Pseudomonas aeruginosa*
1	11	6	4.58	*Khaya senegalensis*	Analgesic (Chest pain)	13	6	6
2	18	4	3.75	*Garcinia cola, Citrus aurantifola, Bidens pilosa*	Anticough	15	10	8
3	20	4	6.35	*Costus afer, Carica papaya*	Anticough	17	7	16
4	24	4	4.85	*Citrus aurantifola, Morinda lucida*	Antiacid, Antimalaria	11	20	14
5	25	3	5.03	*Telferia occidertale*	Antianemic	9	10	15
6	27	2	4.53	*Spondias mombin, Citrus aurantifola*	Antinfective (STIs)	19	6	12
7	30	3	6.38	*Hedranthera bartesi, Cymbogon citrates, Mangifera indica*	Antinfective (STIs), Antibacterial	14	15	13
8	42	3	5.70	*Pterocarpus soyauxii, Talinum spp, Citrus medica*	Anticonstipation	9	14	20
9	58	4	5.30	*Olax subsceroidea, Citrus medica, Zingiber officinale*	Antiimpotence	16	20	11
10	59	4	6.76	*Aloe vera, Cola nitida, Citrus aurantifola*	Antifever	13	16	8
11	62	1	5.22	*Aspilia africana, Cymbogon citrates*	Nervous disorder, Prophylaxis	16	7	7
12	73	4	4.83	*Spondias mombin, Ceratotheca spp, Cimelina arborea,Vernonia spp*	Antihypertensive Antitumor, Antibacterial	10	8	10
13	81	6	5.65	*Musanga cecropioides, Senna alata*	Antibiotic	14	10	9
14	86	1	6.09	*Xylopia aethiopica, Crassocophalum spp, Jatropha curcas, Allium sativum*	Antiacid	6	14	14
15	91	1	4.54	*Erythrina senegalensis, Asmina triloba, Zea mays*	Antinfective (Kidney infections)	9	13	15
16	103	4	6.59	*Elaesis guineense, Apium gravedems, Citrus limon*	Antifever, Antitonsilitis , Antirhematic	11	9	15
17	112	8	6.05	*Momordica characxtia, Baphia spp*	Aphrodisiac , Antibiotic	13	9	11
18	125	3	6.35	*Aspillia africana, Hymenocardia spp, Acadirachta indica*	Antidysenteric, Antinfective	10	8	16
19	136	8	6.56	*Pyenanthus spp, Talinum triangulase*	Antihypertensive	18	15	18
20	146	3	4.77	*Trichilia spp, Mucuna spp, Terminalic catappha*	Antianemic, Antidepressant	19	13	14

Table 3: Inhibitory Zone Diameter (mm) Of Some Liquid Herbal Preparations and Conventional Antibiotics

		Standard Inhibitory Zone Diameter (mm) Of Conventional Antibiotics							
Test Isolates	**Mean IZD (mm)**	**CIP**	**ERY**	**GEN**	**OFL**	**AML**	**CTR**	**CH**	**CAZ**
Staphylococcus aureus	15.25 ±0.35^{e}	28.00 ±1.41^{bc}	30.50 ±0.70^{ab}	28.00 ±1.41^{bc}	28.50 ±0.71^{bc}	33.00 ±1.41^{a}	28.50 ±0.70^{ab}	25.50 ±0.70^{c}	20.50 ±0.70^{d}
Escherichia coli	16.25 ±0.35^{e}	36.50 ±0.70^{a}	13.50 ±0.70^{f}	28.50 ±0.70^{c}	31.50 ±0.71^{b}	23.50 ±0.70^{d}	22.50 ±0.70^{d}	27.50 ±0.70^{c}	33.50 ±0.70^{b}
Pseudomonas aeruginosa	13.25 ±0.35^{d}	30.50 ±0.70^{a}	0.00	24.50 ±0.70^{b}	23.50 ±0.70^{b}	0.00	20.50 ±0.70^{c}	0.00	32.50 ±0.70^{a}

Sample means with the same superscripts across the row shows that there was no significantly difference (P>0.05) while means with different superscript across the row shows that there was a significant difference (P<0.05).

DISCUSSION

There are increasing interests in the antibacterial properties of medicinal herbs. This is particularly so due to increased antibiotic resistance observed with conventional drugs.

This study assessed the phytochemical qualitatively in each LHP. Alkaloids were detected in 47 (30%) samples and may be responsible for the good antibacterial activity demonstrated by some LHPs. This is less if compared to the values *i.e* 70% and 61% alkaloid contain in herbal medicine studied by Al Jamal *et al.,* (2018); Abba *et al.,* (2009).

Quinones occurred only in 9(6%) LHPS being the least occurring phytochemical and is less compared to 10% and 56% qualified by Al Jamal *et al.,* 2018 and Abba *et al.,* 2007. 11(7%) LHPs were assessed to contain saponins and is less when compared to findings of 20% and 59% reported by Abba *et al.,* (2009); Al Jamal *et al.,* (2018). Thirty (19%) LHPs tested also indicated the presence of flavonoids which may be partly responsible for the medicinal properties of some LHPs. This contrast with 52% derived flavonoids content reported by Abba *et al.,* (2009).

Glycosides were identified in 27 (17%) LHPs and has been shown to possess antimicrobial activity. Sequestered glycosides when hydrolyzed by glycosidase yield more active aglycones that may be oxidized to highly reactive free radicals creating an extremely hostile environment for developing pathogens (Dean *et al.,* 1987). This is less to 90% and 35% glycoside content in the findings of Abba *et al.,* (2012); Al Jamal *et al.,* (2018).

Tannins was the second most occurring phytochemical, detected in 34 (22%) LHPs. This contrast with findings of 64% and 60% tannin content from herbal medicine studied by Abba *et al.,* (2012) and Al Jamal (2018).

Invitro antibacterial activities of these LHPs may be due to the chemical nature of the active phytoconstituents or diluents, which specifies their modes of action on bacterial cells as well as their therapeutic uses. Mean IZD of *S. aureus, P. aeruginosa* and *E. coli* to LHPs were 13.1mm, 12.0mm and 11.5mm respectively. This differs with the findings of Agbo *et al.,* (2012) and Ujam *et al.,* (2013).

Further study should be conducted to determine the concentration of the LHPs that could be explored to show their therapeutic effects in the traditional management of different infections.

CONSENT

Not Applicable

ETHICAL APPROVAL

Not Applicable

COMPETING INTEREST

Authors Have No Competing Interest to Declare

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