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ASSESSMENT OF INVITRO ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL CONSTITUENTS OF TWO COMMERCIAL HERBAL PREPARATIONS SOLD IN JOS

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ABSTRACT

The use of herbal medicaments is an old tradition globally and in Nigeria, but the efficacies of these preparations are not adequately confirmed and reported. The in-vitro antibacterial efficacy of two commercial herbal preparations was assessed, to ascertain their antibacterial efficacy and used as cheap alternative treatment for various ailments. Swedish (SB) and Living Bitters (LB) were obtained from Pharmacy shops in Jos, and were tested against six (6) selected Gram positive and Gram negative bacteria using the agar diffusion technique. Susceptibility was measured by inhibition zones for different concentrations of the preparations against selected organisms. *Salmonella typhi* was most susceptible with zones of inhibition of 37mm and 33.7 mm for SB and LB respectively. Similarly, *Staphylococcus aureus* produced zones of inhibition of 37mm and 33.4mm for the two suspensions. There was a dose dependent increase in diameters of zones of inhibition. The Minimum Inhibitory Concentration (MIC) for both SB and LB were 50% (*S. typhi*) and 25% (*S. aureus*) of the original concentrations respectively. The Minimum Bactericidal Concentration (MBC) for SB and LB were 50% (*for S. typhi*) and 25% (*for S. aureus*) respectively. *E. coli* was moderately sensitive while *Pseudomonas aeruginosa* was resistant. Phytochemical constituents identified in both herbal suspensions confirmed presence of bioactive compounds such as flavonoids, saponins and phenols. The flavonoid concentration in SB was higher (++) than the amount in LB (+). More studies should be carried out to ascertain the usefulness of these herbal products as alternative treatment for clinical diarrheal cases and wound or skin lesions.

INTRODUCTION

The abuse of use of antimicrobial agents has resulted in an increased emergence of drug resistant bacteria and this has necessitated the need for alternative treatment. For example, *Staphylococcus aureus* resistance to methicillin, vancomycin have been reported in various hospitals in Kuwait and India (Udo *et al.*, 2008; Aqil, *et al.*,2005) and multidrug resistant Shigella species are reported in Nigeria (Iwalokun *et al.*, 2001). These resistant bacteria were shown to have responded to various medicinal plant products. In assessing the antibacterial effectiveness of medicinal plants, Chah *et al.*, (2006) evaluated the methanolic extracts of *Ageratum conyzoides*, *Anthoclesistad jaloniensis*, *Napoleona imperialis*, *Ocimum gratissimum* and *Psidium guajava* on wound isolates of standard isolates of *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Proteus* spp. and *Shigella* spp. using the agar diffusion method. *Psidium guajava* and *Anthocleistad jalonensis* extracts prevented growth of 81.8 and 72.7% of the test organisms, respectively.

Over time various, plant products have been tested and found to show promise to treat even fungal infections (Alade, *et al.*,1993; Nwosu *et al.*,1995; Nkuo-Akenji *et al.*,2001; Ajose, 2007; Adetutu *et al.*,2011).Several plants have been found to have dermatologic importance in Nigeria; this was demonstrated by a survey which elaborated the impulsive use of herbal preparations which conforms to clinical, scientific and pharmacologic tendencies (Ajose, 2007).

Evidently, there are not enough scientific studies that confirm the antimicrobial properties of oral suspensions that are consumed commercially. This study looks into the in vitro antimicrobial activity of the two oral herbal preparations against selected pathogenic microorganisms that are common causes of infection in Nigeria and also assesses the bioactive phytochemical compounds inherent.

MATERIALS AND METHODS

Samples

The herbal products Swedish bitters® and Living Bitters® which are commercially available, were purchased from pharmcy stores. These substances were screened against a total of five (5) bacterial strains.

Determining level of antimicrobial activity

Microorganisms used

The test organisms include, *Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), Klebsiella* spp., *Salmonella typhi* (ATCC 18804), and *Pseudomonas aeruginosa (ATCC 27853).* These were obtained from the Department of Pharmaceutical Microbiology, University of Jos; and National Veterinary Research Institute (NVRI), Vom, in Jos, Plateau State.

Culture Media
Mueller Hinton Culture (Oxoid®) medium was used for *in vitro* anti-microbial assay of the bacterial isolates. This media is described as the standardized medium for in vitro anti-bacterial activity determination (Prescott, 2005). For other processes various culture media were used such as, Nutient broth (LabM®), and Nutrient agar (LabM®). All the culture media were prepared and treated according to manufacturer,s guidelines.

Inoculum
The microorganisms were inoculated into Nutrient broth and incubated at 35 ± 2°C for 4 h. The turbidity of the resulting suspensions was diluted with nutrient broth to obtain a transmittance of 25.0 % at 580 nm. That percentage was found spectrophotometrically comparable to 1 McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0×10^8 CFU/ml. The Bausch & Lomb® spectrophotometer, Model Spectronic 20 was used to adjust the transmittance of the working suspensions.

Stock solution
The test compounds which are composed of several herbs was diluted into three different stock solutions for the first stage of the test; level of antimicrobial activity. This was diluted using ethanol to concentrations that were 50% and 25% of the original product; thus constituting 100% i.e. the pure compound, the compound at 50% and 25% concentrations. These were stored in sterile bottles and allowed to sit at room temperature for 9 days.

Agar diffusion assay
The dilution susceptibility technique was used in evaluating the anti-microbial susceptibility activity. *In vitro* antibacterial activity was evaluated using Mueller Hinton agar (MHA) prepared according to manufacturer's specification; the pure standardized bacterial stock culture was added to agar medium and then plated out, shaken uniformly and allowed to set. A stainless steel borer of diameter 5mm was then used to aseptically bore four holes through the agar medium. Three of these holes bored were filled with 30ul of various concentrations of 100%, 50% and 25% of the herbal preparations; Swedish Bitters® and Living Bitters®, while the last hole was filled with the positive control antimicrobial agent (Ampicillin). The setup was allowed to sit for at least 30 minutes for the herbal preparations to diffuse through the agar medium. These plates were then incubated at 37°C for about 20hours. The zones of inhibition were measured using a transparent ruler. This procedure was repeated in triplicates for each of the six isolates.

Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC)
The MIC of each herbal preparations was evaluated using the broth dilution method as described by Cheesbrough (2002). This was done for the various concentrations of 100%, 50% and 25%. In determination of the Minimal bactericidal concentration (MBC), the test tubes without evidence of growth, (lacking turbidity) were sub-cultured on nutrient agar plates. The lowest concentration of the herbal product(s) setup resulting in no growth after subculturing for 18 to 24 hours, were noted as having the MBC value.

Phytochemical Screening
This was carried out to determine the presence of the biochemical constituent compounds inherent in the preparations. This was done using techniques described by Oboh *et al.*, (2008). To test for phenols, 2.0ml of the herbal preparations was measured into a test-tube, 2.0ml of Ferric Chloride was added. A deep bluish-green solution indicated the presence of phenols. In testing for Balsam, three drops of alcoholic Ferric Chloride was added to 2.0ml of the test substance in tubes. When warmed slightly in a water bath, a dark green coloration indicated the presence of balsam. To test for renins, 2.0ml of sample was measured into a test tube, 2.0ml of acetic anhydride and 2 drops of concentrated sulphuric acid was added. The formation of a violet coloration confirmed the presence of renins. In testing for anthracenes, 2.0ml of the test substance was measured into the testube, 2.0ml of Chloroform was added and shaken. The formation of a brick red colouration confirmed the presence of anthracenes. In testing for flavonoids 2.0ml of the extracts was transferred into a test tube, 10% lead acetate solution was added to the test substance. A creamy or light yellow coloration indicated the presence of Flavonoids. To test for saponins 5.0ml of distilled water was used to dilute 4.0ml of the test substance and shaken vigorously for 2 minutes. Persistent frothing confirmed the presence of saponins. To test for tannins, 4.0ml of distilled water was added to 1.0ml as the test substance of the extract and a few drops of 10% Ferric Chloride solution was added to the mixture and observed for the formation of a blue or green precipitation or coloration, which confirms the presence of tannins. In testing for alkaloids, 1% Hydrochloric acid was added to 2.0ml of the test substance, also 3 drops of Dragendorf reagent was added, the formation of an orange coloration confirmed the presence of alkaloids. Finally in testing for cardiac glycosides, 2.0ml of the test substance was diluted with 2.0ml of Chloroform, 3.0ml of sulphuric acid was carefully added to form a lower layer. A reddish-brown color at the interphase indicates the presence of cardiac glycosides.

RESULTS
The antimicrobial activity of the herbal preparations was performed in triplicates thus the results reflected average values of activity. The herbal preparations; Swedish Bitters® and Living Bitters® inhibited the growth of all the test organisms to different degrees. In Table 1, Swedish Bitters® showed no considerable effect on *Pseudomonas aeruginosa* with inhibition zone of <12mm in both cases. This activity was similar to that of Living Bitters® with inhibition zone

diameter of 13.3mm. The activity of these herbal preparations was quite inhibitory to *Salmonella typhi, Staphylococcus aureus*, and *Klebsiella pneumonia (> 13 mm diameter zone of inhibition).*

The minimum inhibitory concentration (MIC) of both herbal preparations; Swedish Bitters and Living Bitters on the test organisms is shown in Table 2. The MIC of Swedish Bitters was 25% for *Staphylococcus aureus* and *Escherichia coli*, 50% for *Salmonella typhi* and *Klebsiella pneumoniae* and 100% for P*seudomonas aeruginosa*. Table 3 shows the activity of Living Bitters ®.

In Table 4, the minimum bactericidal concentration, (MBC) of Swedish Bitters® is reported to be 1650µg/ml for *Staphylococcus aureus*, 3300µg/ml for *Escherichia coli, Klebsiella pneumoniae*, and *Salmonella typhi* and for *Pseudomonas aeruginosa* it was 6600µg/ml. for living bitters the MBC was 25% for *Staphylococcus aureus*, 50% for *Salmonella typhi* and *Escherichia coli*, while that of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was 100% i.e. in view of the concentration of the original preparation as reported in Table 5.

The Phytochemical screening reveals that both herbal preparations have flavonoids, saponins and phenols. In addition Swedish Bitters has tannins and living bitters has alkaloids. This is shown in Table 6.

Table 1: Susceptibility of bacterial isolates to Swedish bitters and Living bitters

		Zones of Inhibition (mm)						
		Swedish Bitters® %(v/v)			Living Bitters® %(v/v)			
S/No.	Organism(s)	100% 6600µg/ml	50% 3300µg/ml	25% 825µg/ml	100%	50%	25%	*Positive control
1.	*Salmonella typhi*	37	22.7	11	33.7	20.3	9	38
2.	*Staphylococcus aureus*	37	28	11.5	34.6	24.7	8.5	34
3.	*Klebsiella pneumoniae*	17.5	10.3	5.5	14	8	NR	22
4.	*Escherichia coli*	14.7	8.7	NR	14.3	8.7	NR	16.7
5.	*Pseudomonas aeruginosa*	13.3	10.7	NR	11	8	3	17

NR= Not recorded, v/v = volume per volume proportions, mm = millimeters,* = Ampicillin

Table 2: Minimum Inhibitory Concentration of Swedish Bitters

Concentration of the Herbal Preparation					
S/No	Microorganisms	100%	50%	25%	Positive control
1	*Salmonella typhi*	-	-	+	-
2	*Staphylococcus aureus*	-	-	-	-
3	*Klebsiella pneumoniae*	-	-	+	-
4	*Escherichia coli*	-	-	-	-
5	*Pseudomonas aeruginosa*	-	+	+	-

SB= Swedish Bitters, - (no growth), + (Bacterial growth)

Table 3: Minimum Inhibitory Concentration (MIC) of Living Bitters

Concentration of the Herbal Preparation					
S/No	Microorganisms	100%	50%	25%	Positive control
1	*Salmonella typhi*	-	-	+	-
2	*Staphylococcus aureus*	-	-	-	-
3	*Klebsiella pneumoniae*	-	+	+	-
4	*Escherichia coli*	-	-	-	-
5	*Pseudomonas aeruginosa*	-	+	+	-

- (no growth), + (bacterial growth)

Table 4: Minimum Bactericidal Concentration (MBC) of Living Bitters

Concentration of the Herbal Preparation					
S/No	Microorganisms	100%	50%	25%	Positive control
1	*Salmonella typhi*	+	+	x	x
2	*Staphylococcus aureus*	+	+	+	x
3	*Klebsiella pneumoniae*	+	x	x	x
4	*Escherichia coli*	+	+	x	x
5	*Pseudomonas aeruginosa*	+	x	x	x

+ (Bacterial growth), x (Absence of growth)

Table 5: Minimum Bactericidal Concentration (MBC) of Swedish Bitters

		Concentration of the Herbal Preparation			
		100%	50%	25%	
S/No	Microorganisms	6600µg/ml	3300µg/ml	1650µg/ml	Positive control
1	*Salmonella typhi*	+	+	x	x
2	*Staphylococcus aureus*	+	+	+	x
3	*Klebsiella pneumoniae*	+	+	x	x
4	*Escherichia coli*	+	+	-	x
5	*Pseudomonas aeruginosa*	+	x	x	x

+ (Bacterial growth), x (Absence of growth)

Table 6: Phytochemical properties of Swedish and Living Bitters

S/No	Phytochemical Bioactive Compound	Swedish Bitters®	Living Bitters®
1.	Phenols	+	+
2.	Balsam	-	-
3.	Renins	-	-
4.	Anthracenes	-	-
5.	Flavonoids	++	+
6.	Saponins	+	+
7.	Tanins	+	-
8.	Alkaloids	-	+
9.	Cardiac glycosides	-	-

Key: Presence = +; Absence = -

DISCUSSION

The results show the antimicrobial activity against *Salmonella typhi, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* to different degrees by both herbal preparations. This agrees and provides a scientific description of the history of Swedish bitters which is thought of as a cure for several intestinal ailments. The inhibitory effects of these herbal preparations can be attributed to the bioactive compounds; Saponins, tannins, Flavonoids, phenols and alkaloids. Aruda *et al.,* (2011) evaluated the bark from Jacaranda cuspidifolia Mart. (Family Bignoniaceae), a Brazilian medicinal plant that is traditionally used as anti-syphilis and anti-gonorrhea treatment. They found the phytochemical constituents to contain saponins, coumarins, flavonoids, tannins, quinones, alkaloids among others and attributed the antibacterial activity of the plant to these constituents. In a similar study, Ndjonka *et al.,* (2011) established the presence of flavonoids, alkaloids, saponins, carbohydrates and tannins in the extracts of various Cameroonian and Ghanaian medicinal plants, used in the control of helminthes infections.

In our study, Swedish Bitters® showed higher inhibitory activity to the test organisms than Living Bitters®; the differences in inhibitory effect of these compounds can be attributed to the varying component herbs and bioactive compounds noted in the course of this study.

The antimicrobial activities of Swedish Bitters and Living Bitters provide information on the relevance of new and less toxic alternative treatments. Furthermore this study suggests effective synergic action of these herbal preparations. Similar studies of this kind would support the development of herbal products indigenous to Nigeria. Further studies on the clinical importance of these herbal preparations will further validate the importance of these preparations as alternative antimicrobial agents.

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REFERENCES

Adetutu A, Morgan WA, Corcoran O. (2011). Antibacterial, antioxidant and fibroblast growth stimulation activity of crude extracts of *Bridelia ferruginea* leaf, a wound-healing plant of Nigeria.*J Ethnopharmacol. Jan 7;133(1):116-9. Epub 2010 Sep 21.*

Ajose FO. (2007). Some Nigerian plants of dermatologic importance. *Int J Dermatol. Oct;46 Suppl 1:48-55.*

Alade PI, Irobi ON. (1993). Antimicrobial activities of crude leaf extracts of *Acalypha wilkesiana.J Ethnopharmacol. Aug;39(3):171- 4.*

Aqil F, Khan MS, Owais M, Ahmad I. (2005). Effect of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin resistant *Staphylococcus aureus. J Basic Microbiol.45(2):106-14.*

Chah KF, Eze CA, Emuelosi CE, Esimone CO. (2006). Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants.. *J Ethnopharmacol. 2006 Mar 8;104(1-2):164-7. Epub 2005 Oct 13.*

Irobi ON, Banso A. (1994). Effects of crude leaf extracts of Acalyphatorta against some anaerobic bacteria.*J Ethnopharmacol. Jun;43(1):63-5.*

Iwalokun BA, Gbenle GO, Adewole TA, Smith SI, Akinsinde KA, Omonigbehin EO. (2003). Effects of *Ocimum gratissimum L* essential oil at sub-inhibitory concentrations on virulent and multidrug-resistant Shigella strains from Lagos, Nigeria.*APMIS. Apr;111(4):477-82.J Dent. Jun;5(2):123-7.*

Nkuo-Akenji T, Ndip R, McThomas A, Fru EC. (2001). Anti-Salmonella activity of medicinal plants from Cameroon.*Cent Afr J Med. Jun;47(6):155-8.*

Oboh IE, Obasuyi O, Akerele J.O. (2008). Phytochemical and comparative antibacterial studies on the crude ethanol and aqueous extracts of the leaves of *Lecaniodis cuscupanoides* Planch (Sapindaceae).*Acta Pol Pharm. Sep-Oct;65(5):565-9.*

Okoli AS, Iroegbu CU. (2004). Evaluation of extracts of *Anthocleistad jalonensis, Nauclea latifolia* and *Uvaria afzalii* for activity against bacterial isolates from cases of non-gonococcalurethritis.*J Ethnopharmacol.; 92(1):135-44.*

Udo EE, Al-Sweih N, Dhar R, Dimitrov TS, Mokaddas EM, Johny M, Al-Obaid IA, Gomaa HH, Mobasher LA, Rotimi VO, Al-Asar A. (2008). Surveillance of antibacterial resistance in Staphylococcus aureus isolated in Kuwaiti hospitals. *Med Princ Pract.;17(1):71-5.*

Arruda AL, Vieira CJ, Sousa DG, Oliveira RF, Castilho RO.(2011).Jacaranda cuspidifolia Mart.(Bignoniaceae) as an antibacterial agent. *J Med Food. Dec;14(12)*:1604-8. Epub 2011 Jun 11.

Ndjonka D, Agyare C, Lüersen K, Djafsia B, Achukwi D, Nukenine EN, Hensel A, Liebau E. (2011). In vitro activity of Cameroonian and Ghanaian medicinal plants on parasitic (Onchocerca ochengi) and free-living (Caenorhabditis elegans) nematodes. *J Helminthol. Sep;85(3)*:304-12. Epub 2010 Sep 24.