Nigerian Journal of Pharmaceutical Sciences
Vol. 18, No1, 2019, ISSN: 0189-823X

CHARACTERIZATION, ANTI-INFLAMMATORY AND ANTIMICROBIAL POTENTIALS OF A HERBAL PREPARATION

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ABSTRACT

Herbal product (HP) (from *Ricinus communis* and honey) is used locally for the management of inflammatory and inflammation related disorders including asthma. The current study was carried out to determine the phytochemicals, total phenolic and flavonoid contents, chemical profile, elemental components, anti-inflammatory and antimicrobial activity of the HP used for treatment of asthma. Total phenolic and flavonoid contents were determined by the Folin-Ciocalteus reagent and $AlCl_3$ colorimetric method respectively. The chemo-profiling was done using TLC. Elemental analysis was determined using Atomic Absorption Spectroscopy (AAS). Albumin-induced paw oedema model was used to determine the anti-inflammatory activity while agar well diffusion method was used to determine the antimicrobial activity of the HP at different concentrations. Flavonoids, saponins, alkaloid, cardiac glycoside, steroids and terpenoids were detected in the HP. Total phenolic and flavonoid contents obtained were 10.00 mg/g gallic acid equivalent and 336.60 mg/g quercetin equivalent respectively. The chemo-profiling showed one spot at 254 nm but none at 366 nm. AAS indicated the presence of K, Ca, Fe, Na, Zn, Cu and Pb (Cu and Pb were within WHO acceptable limit). The 100 % concentration produced sudden but transient significant reduction in oedema size at 30 minutes. However, at 60 minutes the effect produced at 25 % was comparable to that of Ibuprofen at 20 mg/kg body weight. At 100% concentration HP all the organisms tested were sensitive except for *Pseudomonas aeruginosa*. The presence of flavonoids may justify the anti-inflammatory and antimicrobial activities thus validating its use in local treatment of Asthma

Keywords: Herbal product, Characterization, Asthma, anti-inflammatory, antimicrobial.

INTRODUCTION

Asthma is a common chronic inflammatory disease of the airways (Masoli *et al.,* 2004) which adversely affects normal lung function. It's characterized by reversible airflow obstruction and bronchospasm. Common symptoms include wheezing, coughing, chest tightness, and shortness of breath (AAFA, 2015). Asthma affects about 235 million people world-wide and over 80% of asthma deaths occurs in low and lower-middle income countries (WHO, 2015).

Herbal medicine is more accessible to most of the population. About 60 to 85% of the populations of every country of the developing world rely on herbal or indigenous forms of medicine (Onyeka *et al.,* 2012). The reasons for the high patronage of herbal medicine are the high cost of very effective antibiotics and the problem of antibiotic resistance which is very common in developing countries (Hack, 2006).

In Kwara state, Nigeria, locals consumed polyherbal formulations against asthma, stomach and inflammation disorder as well as other reasons. One of these formulations is a mixture of honey and oil from *Ricinus communis*. This herbal product which is brown in colour and smells like honey is a polyherbal formulation used locally for the management of inflammation disorders especially asthma. The present study is to carry out chemical and antimicrobial studies on the herbal product (HP). The study would also examine scientifically the folkloric claim in the use of the HP for treatment of asthma.

MATERIALS AND METHODS

Materials

The following materials were used: Normal saline, Ibuprofen, Quercetin, Garlic acid, $AlCl_3$ (Sigma, UK), white albino rats, Stop watch (China), Albumin (Sigma, UK), analytical weighing balance (Ohaus, USA). Thin layer chromatographic plates (MERCK, silica gel 60 F_{254} 0.2 mm), Atomic Absorption Spectroscopy (GBC Avanta Model, Australia), nutrient agar, Microbact GMB 24E kits, Muller Hilton Agar, Folin Ciocalteu reagent (Sigma).

Methods

Herbal product

The HP was collected from an herbal practitioner at Offa, Kwara State, Nigeria in 2015 and stored at room temperature.

Solubility of HP

The solubility was determined using petroleum ether, chloroform, ethyl acetate, acetone, ethanol, methanol and distilled water. Using a test tube, 5 mL each of the solvent was added to 0.1 g of the HP and vortex mixed, then observed if it dissolves.

Preliminary Phytochemical screening of HP

Basic phytochemical screening to detect the presence or absence of plant chemical constituents such as alkaloids, tannins, saponins, anthraquinones, flavonoids, carbohydrates, cardiac glycoside, steroids and triterpenes were carried out on the aqueous HP using standard procedures (Brain and Turner, 1975; Evans, 1989; Sofowora, 1993).

Chemo-profile of HP

Normal phase analytical TLC was performed using silica gel 60 F_{254} precoated plate (0.2mm). The HP was spotted on a pre-treated TLC silica gel plate 60 F_{254} (from Merck, Germany) and the plate developed at a distance of 10 cm using CH_3OH: EtOAc: $CHCl_3$: Pet-ether (3:2:1:0.5) as mobile phase. After derivatization the plate was

allow to dry at room temperature (28 ^{o}C), and examined under ultra violet light at 254 nm and 366 nm; after which it was sprayed with 5%-sulphuric acid reagent. It was dried in an oven for 5min at 120 ^{o}C. The Rf values of the spots were measured (Mishra *et al.,* 2013).

Determination of total phenolic and flavonoid contents

Total phenolic content of HP was determined by the Folin–Ciocalteu method (Meda *et al*., 2005). Aliquots of 0.1 g HP was dissolved in 1 ml deionized water. This solution (0.1 ml) was mixed with 2.8 ml of deionized water, 2 ml of 2% sodium carbonate (Na_2CO_3), and 0.1 ml of 50% Folin–Ciocalteau reagent. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 750 nm against a deionized water blank on a spectrophotometer (Hitachi, Model 100-20). Gallic acid (GA) was chosen as a standard. The levels of total phenolic contents in HP was determined in triplicate. The data were expressed as milligram gallic acid equivalents (GAE)/g aqueous HP.

The total flavonoid content was determined using colorimetric method (Ebrahimzadeh et al., 2001). 0.5 ml solution of the HP in methanol was mixed with 0.5 ml of 2% aluminum chloride and left at room temperature for one hour, thereafter, the absorbance was measured at 420 nm. The HP was evaluated at a final concentration of 0.1 mg/ml. Quercetin was chosen as a standard. The levels of total flavonoid contents in HP was determined in triplicate. The data were expressed as milligram quercetin equivalents (QE)/g aqueous HP.

Elemental analysis

Eleven Elements namely Cu, Zn, Co, Mn, Mg, Fe, Cr, Ca, K, Na, and Pb were assessed quantitatively according to the method of Association of Official Analytical Chemist (AOAC, 1980) using Atomic Absorption Spectrometer (AAS). Standards and digested sample were aspirated and the mean signal responses were recorded at each of the element respective wavelengths.

Anti-inflammatory activity

The model used for the experiment is the egg albumin-induced paw edema model in rats (Winter *et al.,* 1962). The preparations were administered orally at 100%, 50% and 25% concentration, while 10 ml normal saline/kg body weight and 20 mg ibuprofen/kg body weight were administered orally to negative and positive controls respectively. Thirty minutes after extract administration, inflammation was induced by subplantar injection of 0.1 ml of fresh undiluted egg albumin. Edema was assessed in terms of volume of distilled water displaced by the paw before and at 0.5, 1, 1.5, 2, 2.5 and 3 hours after induction of inflammation.

Antimicrobial activity

Collection, purification, confirmation and storage of the test organisms

Bacterial isolates obtained include *Staphylococcus aureus*, *Escherichia coli*, *Citrobacter freundi, Pseudomonas aeruginosa, Salmonella* species *and Klebsiella oxytoca.* These organisms were purified by repeated sub – culturing on Nutrient agar, characterized by standard bacteriological methods (Cheesbrough, 2004) and the Gram – negative bacteria were further identified using Microbact GMB 24E Kits. The stock cultures were stored at 4 ^{o}C in nutrient agar slants until required.

Standardization of inocula

Bacterial inocula were prepared using the McFarland turbidity standard which gives turbidity scale of approximate bacterial density of 1.2 x 10^8 CFU/ml in accordance

to the method described by the National committee for clinical laboratory standards (NCCLS, 1998).

Sensitivity screening

This was done following the method described by Perez *et al* (1990). Wells were bore into Muller Hilton Agar (MHA) plates using sterile No. 6 cork borer after which the bottom of the wells were sealed using molten MHA. The inocula of each of the test organisms were streaked on the MHA plates aseptically in a laminar flow carbinet. Subsequently, 100µl each of 100%, 200mg/ml, 100mg/ml and 50mg/ml concentrations of the herbal product were separately introduced into the well of the agar culture. The plates were allowed to stand for 1 hour to allow diffusion of the herbal product to take place and then incubated at 37^{o}C for 24 hours.

The antibacterial susceptibility testing of herbal product was also compared with standard antibiotics discs of ciprofloxacin (CIP), tetracycline (TET), amoxicillin (AML), chloramphenicol (CHL) and nitrofurantoin (F) using disc agar-well diffusion method, Cheesbrough (2004). All plates were at 37^{o}C for 24 hours and zones of inhibition were recorded to the nearest millimeter (mm).

Data analysis

Results were presented as mean ± standard error of mean. The zones of inhibition were measured and compared with Clinical Laboratory Standard Institute, CLSI, 2014 standard. Activity index was also used to compare antibacterial activity with the most effective antibiotic. Statistical analysis was done using One Way ANOVA and values at $P<0.05$ were taken as significant.

RESULTS

The solubility profile and the preliminary phytochemical test results are shown in Tables 1 and 2 respectively. Total phenolic and flavonoid content in aqueous HP**,** the results of elemental analysis and Anti-Inflammatory Activity of HP are shown in tables 3, 4 and 5 respectively.

Table 1. Solubility profile of HP

Solvents	solubility
Petroleum ether	Insoluble
Chloroform	Insoluble
Ethyl acetate	Insoluble
Acetone	Insoluble
Methanol	Sparingly soluble
Ethanol	Sparingly soluble
Water	Soluble

Table 2. Phytochemicals present in the aqueous solution of HP

Constituent	Tests	Inference
Tannins	Pb subacetate	-
	$FeCl_3$	-
Flavonoids	Shinoda	+
	NaOH	+
Alkaloids	Meyers	-
	Wagners	-
	Dragendoff	-
Cardiac glycoside	Kella-Killiani	+
Saponins	Frothing	+
Unsaturated steroids and Triterpenes	Liebermann' burchards	+

Table 3. Total Phenolic and Flavonoid Content in Aqueous HP

Sample	Phenolic content	Flavonoid content
HP	10.00 mg/g gallic acid equivalent	336.60 mg/g quercetin equivalent

Table 4. Quantitative Analysis of Heavy and Essential Metals in the Recipe

S/N	ELEMENT	CONCENTRATION (µg/ml)
1	Lead (Pb)	ND
2	Cobalt (Co)	ND
3	Copper (Cu)	1.446
4	Zinc (Zn)	11.65
5	Sodium (Na)	121.5
6	Iron (Fe)	49.276
7	Calcium (Ca)	ND
8	Potassium (K)	290.2
9	Manganese (Mn)	1.3466
10	Chromium (Cr)	ND
11	Magnesium (Mg)	264.538

Keys: Not Detected = ND

Table 5. The Change in Paw Oedema assessed at different Time Intervals for Anti-inflammatory activity of HP

Time (Min)	100% Conc	50% Conc	25% Conc	20mg/kg Ibuprofen	Control
30	84%	-5%	-10.00%	-45%	-
60	-5.60%	10.40%	15.09%	15.09%	-
90	-17%	-7.55%	-4.35%	-8.69%	-
120	-41.66%	-33.33%	-27.77%	-44.44%	-
150	-50.00%	-17.95%	-15.38%	-17.95%	-
180	-61.00%	-17.14%	-14.29%	-17.14%	-

Antimicrobial activity

Table 6 showed the susceptibility profile of herbal product to selected bacteria isolates. At 100% concentration, herbal product showed activity against all isolates except for *P. aeruginosa* which was resistant. With *S. aureus,* sensitivity was observed at 100mg/ml while with the other Gram – negative organisms at 200mg/ml and generally resistant at 50mg/ml. Table 7 showed the zones of inhibition of herbal product as compared with standard antibiotics. Using CLSI, 2014, *S. aureus* was susceptible to all the antibiotics used and herbal product had activity index of 0.96 when compared with nitrofurantoin. *E. coli, Salmonella* spp, *Citrobacter freundi,* and *P. aeruginosa* were resistant to TET, AML and CHL. *K. oxytoca* was susceptible to TET, CHL and F while *Ps. aeruginosa* was susceptible to only CIP. The herbal product had good activity index (1.0) against *E.coli* with respect to nitrofurantoin while its activity index was 0.00 with *Ps. aeruginosa* with respect to ciprofloxacin.

Table 6: Susceptibility Profile of Herbal Product to Selected Isolates

Isolates	Concentrations (mg/ml)			
	100%	200	100	50
S. aureus	+	+	+	-
E. coli	+	+	-	-
Salmonella spp	+	+	-	-
C. freundi	+	-	-	-
Klebsiella oxytoca	+	-	-	-
Ps. Aeruginosa	+	-	-	-

Keys: Sensitivity = +, Resistant = -

Table 7: Zones of Inhibition Standard Antibiotics as compared with Herbal Product

Organisms	Antibiotics (mm)						Activity Index
	CIP	TET	AML	CHL	F	HP (100%)	
S. aureus	36 (S)	33 (S)	15(I)	32(S)	25(S)*	24	0.96
E. coli	18(R)	10(R)	0(R)	0(R)	18(R)*	18	1.00
Sal. Spp	28(I)*	0(R)	12(R)	0(R)	10(R)	15	0.53
C. freundi	0(R)	0(R)	0(R)	0(R)	21(S)*	8	0.38
K. oxytoca	30(I)	18(S)	0(R)	29(S)	17(S)*	10	0.59
Ps. aeruginosa	32(S)*	0(R)	0(R)	0(R)	0(R)	0	0.00

Key: (*) = Zones used to calculate Activity Index, CIP = ciprofloxacin, TET = tetracycline, AML = Amoxicillin, CHL = chloramphenicol, F = nitrofurantoin, HP = Herbal product, R = Resistant

DISCUSSION

The chemo-profiling of the extract of HP showed only one spot at 254 nm but no spots at 366nm.

The solubility profile (Table 1) shows that most of the components present in the HP were mostly polar components since HP was mostly soluble in water. This was confirmed from Table 2 which showed that the HP contained flavonoids, saponins, alkaloid, cardiac glycoside, steroids and terpenoids. The Chemo-profile showed only one sport at 254nm when viewed under UV lamp. The total phenolic content was found to be 10.00mg/g gallic acid equivalent while the flavonoid content was found to be as high as 336.60mg/g quercetin equivalent (Table 3). This shows that HP contains mostly flavonoids which are polar components. Plants with high polyphenolic contents have higher importance as natural antibiotics (Baravalia *et al.,* 2009). Phenolic compounds possesses properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al.,* 2007).

Plants extract's antioxidant properties have been attributed to their polyphenolic contents (Murthy *et al.,* 2002). Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc (Ali *et al.,* 2008). Flavonoids are ideal scavengers of peroxyl radicals due to their favourable reduction potentials relative to alkyl peroxyl radicals, and thus they are effective inhibitors of lipid peroxidation (Schroeter *et al.,* 2002). This strong anti-oxidative property of flavonoids has made

them protective against airway diseases linked to oxidative stress (Rice-Evans, 2001; Polovka *et al.,* 2003). Several epidemiologic data suggest beneficial effects of flavonoids on asthma. A 30-year longitudinal epidemiological study reported that the incidence of asthma is lower in populations with higher intake of flavonoids (Knekt *et al.,* 202).

Eleven metals were examined in the sample but four of the metals (lead, chromium, calcium and cobalt) where not detected while manganese, zinc, iron, copper, sodium and magnesium were detected, quantified (Table 5 and 6) and were within WHO acceptable limits. Zn, Mn, Mg and Cu are essential elements which play important roles in various cell processes including; normal growth, brain development, utilization of vitamins and neutralization of free radicals.

The change in paw oedema was assessed at different time intervals. The 100 % concentration produced sudden but transient significant reduction in oedema size at 30 minutes. However, at 60 minutes the effect produced by the preparation at 25 % was comparable to the effect of ibuprofen at 20 mg/kg body weight. Oedema slowly builds up in group treated with 25 % of the preparation while the oedema size in other treatment groups was highly significant. The result indicated the enrichment of the herbal medicine with K, Mg, Fe, Na and Zn. The copper and iron in the HP could work together to help the body form and utilize red blood cells. This then keeps block vessels, nerves, the immune system and bones healthy and functional.

Bacterial infections caused by the genus *Staphylococcus* are a great threat to both humans and animals. *S. aureus* spreads pneumonia at slow rates (Simor *et al.,* 2001). *P. aeruginosa* is a common bacterium that can cause diseases in humans; it is most notorious for causing lung infections or pneumonia. If *P. aeruginosa* colonies occur in critical body organs, such as the lungs, urinary tract and kidneys, the results can be fatal (Balcht & Raymond, 1994). *E. coli* causes many infections including gastroenteritis and urinary tract infections, pneumonia, meningitis, bone and joint infections, and skin and soft tissue infections (Todar, 2007). *Salmonella* causes gastroenteritis resulting to typhoid fever; *C. freundi* causes opportunistic infections of the respiratory tract, urinary tract, and the blood (Berkowitz and Umeh, 2011; Whalen *et al.,* 2007).

HPs are of great importance because they can be active against infectious diseases. In this study, varying levels of susceptibility of the herbal product ranging from resistance to moderate and high inhibition was observed (Table 6 and 7). This herbal product showed higher antibacterial activity against *S. aureus* than against the Gram – negative bacteria while *P. aeruginosa* was completely resistant. This is similar to the finding of Jombo and Enenebeaku (2008) who tested antimicrobial activity of *R. communis* and found it to be active against *K. pneumoniae, E. coli, P. vulgaris, and S. aureus*. El-Kamali and El-Amir, (2010) who screened the antibacterial activity and phytochemical properties of eight selected species, reported all extracts showed good antibacterial activity against four strains of bacteria. Estrada (2008) also reported good antimicrobial activity of honey against *S. aureus* (ATCC 25923), *S. epidermidis* (UCR 2902), *P. aeruginosa* (ATCC 9027), *E. coli* (ATCC25922), *S. enteritidis* (ATCC 13076), *L. monocytogenes* (ATCC 19116) and *A. niger* which is also in line with the present study.

CONCLUSION

The results show that the phytochemical bioactive compounds found in the HP could be responsible for its antiasthma and antibacterial activities. The metal content which are within WHO permissible limits implies that the HP may not result to any health hazards to consumers.

The lack of standardized protocol for the preparation of these herbal remedies and also the age of the HP makes it necessary for traditional herbal council and scientists to act as a clearing and regulating outfit to standardize, monitor and regulate the production of HPs.

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