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Antibacterial Activity and GC-MS Analysis of a Traditional Herbal Formulation "Komi Da Ruwanaka" Sold in Katsina, Nigeria

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Abstract
The antibacterial activity of a traditional medicinal formulation also called "komi da ruwanka" used in the treatment of various diseases was assessed by agar well diffusion method. The formulations sampled from three different locations exhibited a significant ($P<0.05$) antibacterial activity against *Staphylococcus aureus*: (KAT1 15.67 ± 0.67, KAT2 15.00 ± 0.58 and KAT3 15.00 ± 0.58). There was no significant ($P>0.05$) antibacterial activity on *Bacillus subtilis*, *Escherichia coli, Salmonella typhi and Shigella flexnerii*.
The content of the formulation was analysed by gas-chromatography coupled with mass spectrometry. Chlorobenzene (16.36%), P-xylene (13.77%), 1-methoxy-2-propyl acetate (11.49%) and toluene (11.39%) were identified as the major components. While methylligrocerate (0.16%), 3,5-Di-tert-butylphenol (0.37%), decane (0.45%), and dimethylglutarate (0.59%) as the minor components. The Nigerian traditional medicine system needs to be properly organized and should be incorporated within a regulatory frame work implemented at the federal level to ensure basic levels of standardization and quality control in the manufacture of traditional medicinal formulations.
Keywords: Antibacterial, GC-MS analysis, traditional formulation, Herbal drug vendors

INTRODUCTION

The global market for herbal medicine is over US$60 billion annually and is growing steadily. Countries in Africa, Asia and Latin America use traditional medicine to help meet their primary health care needs. In Africa, up to 60% of the population use traditional medicine for primary health care (WHO, 2005).
Traditional medicine is the most common system of alternative medicine in developing countries with herbal medicine being the most sought after but according to the World Health Organization (WHO) is the sum total of the knowledge, skills and practices based on theories, beliefs and experiences indigenous to different cultures used in the maintenance of health as well as in prevention, diagnosis, improvement or treatment of physical and mental illnesses (Ernst, 1998).
In the last two decades, there has been an upsurge in the circulation and usage of traditional medicinal preparations in the treatment and or prevention of diseases in Nigeria. This may not be unconnected with the relatively high cost of conventional pharmaceutical drugs, inaccessibility of orthodox medical services to a vast majority of people particularly in the rural areas, prevalence of fake, substandard or counterfeit drugs in the market and the problem of antibiotic resistance which is very common in developing countries (Adenike *et al.*, 2007).In developing nations, access to essential medicines is severely restricted by lack of resources and poverty.
In an attempt to enhance the acceptability of the traditional medicinal preparations, many of the products have been formulated into conventional modern dosage forms such as tablets, capsules, suspensions, solutions and powders (Oyetayo, 2008).
While it's true that developers of traditional medicinal preparations often make bold, outrageous or bogus claims that their products can cure all forms of ailments from headaches to all sorts of bacterial, viral and fungal infections, critics should not be quick to dismiss traditional medicine as quackery since there are Pros as well or Cons of usage (Liao *et al* ., 2008).
Among the numerous advantages of using herbal medicinal preparation is that they tend to be more effective for long standing health complaints. They have fewer side effects and may be safer to use overtime. Another advantage is that of cost and accessibility to most of the population (Oke, 2002).

Herbal medicinal preparations are not without some disadvantages. For sudden serious illness, mainstream medicine still reigns supreme. Herbal medicinal preparations may not treat serious trauma such as broken leg, heart attack or appendicitis as effectively as a conventional doctor using modern diagnostic tests, surgery and drugs (Fabricant and Fansworth, 2001). There is also the risk of self dosing which can be very harmful. Herbal medicinal preparations also interact with medication and it's a practice that remains highly unregulated (Oyetayo, 2008). The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for antimicrobial substances from other sources (Periyasamy *et al.*,2010). Screening of herbal formulations such as "komi da ruwanaka" for antimicrobial property is important for finding alternatives to commonly used antibioitics for therapeutic use/In this research, antibacterial activity of the herbal formulation was carried out against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi and Shigella flexnerii*. GC-MS analysis was also conducted to identify the constituents present in the formulation.

MATERIALS AND METHODS

Collection of samples

Samples of the popular traditional medicinal preparation were bought randomly from herbal drug vendors in Katsina. A total of nine samples were gotten and the bottles of the samples were labeled appropriately and transported to the Microbiology laboratory of Usmanu Danfodiyo University, Sokoto for analysis.

Test microorganisms for study

Bacterial cultures of *Escherichia coli*, *Bacillus subtilis*, *Staphyloccus aureus*, *Shigella flexnerii*, *Salmonella typhi* were obtained from the microbiology laboratory of Usmanu Danfodiyo University Sokoto and used for antimicrobial assay. The bacterial cultures were checked for viability and maintained on nutrient broth at 37^0C. The bacterial isolates were identified and characterized according to standard biochemical methods.

Media used

Nutrient agar (Lab M, International Diagnostic group (IDG) PLC) and Mueller Hinton agar (oxoid) were used for isolation and microbiological screening. All media were prepared according to the manufacturers' specifications.

Antibacterial assay of samples

Antibacterial activity of the formulation was determined by standard agar well diffusion method as described by National committee for clinical laboratory standard (NCCLS,2002). Molten Sterile Mueller Hinton agar was poured into sterile plates and allowed to set before being dried in oven. A sterile cork borer was used to make wells (6mm in diameter) into the agar. A 24 hour broth culture of the test organisms was adjusted to 0.5 MacFarlard standard and sterile cotton Swab was used to inoculate the bacterial suspension by streaking onto the agar. About 0.5ml of the formulation was poured into the wells and allowed to set. Standard antibiotic (tetracycline 10mg/ml) was used as positive control. Incubation of Petri plates was at 37^0C for 24hours after leaving them for 1 hour to enhance diffusion. The assessment of antibacterial activity of the formulation was based on measurement of the diameter of zone of inhibition formed around the well using meter rule (Hugo and Russel,1998).

Gas Chromatography–Mass Spectrometry (GC-MS)

GC-MS analysis was performed with GCMS-QP2010 Plus (Shimadzu, Japan) at National Institute for Chemical Technology, (NARICT). Among which the gas chromatograph was equipped with flame ionization detector (FID). The injection was conducted in split less mode at 250°C for 3 minutes by using an inlet liner of 0.75 mm i.d. to minimize peak broadening. Chromatography separations were performed by using DB-WAX analytical capillary column 30 m ´ 0.25 mm ´ 0.25 mm (J & W Scientific, Folsom, CA) with helium as carrier gas at a constant flow rate of 0.8 ml/min. The oven temperature was programmed at an initial temperature of 60°C for 5 min, followed by an increase of 5°C/min to 140°C (held for 5 minutes), and finally at 10°C/min to 280°C (held for 10 minutes). The temperature of the FID was set to 250°C. MS operating conditions (electron impact ionization mode) was an ion source temperature of 200°C, ionization voltage of 70 eV and mass scan range of m/z 33 – 450 at 2.76 scans/s.

Volatile compound identification and quantification

The chromatography peak identification was carried out by comparing their mass spectra with those of the bibliographic data of known compounds from the WILEY 6 library (Hewlett-Packard Co., Palo Alto, CA) and NIST 98 library (Hewlett-Packard Co. , Palo Alto, CA) mass spectral database on the basis of the criterion similarity (SI)>800 (the highest value is 1,000).

According to the method (Wanakhachornkrai and Lertsiri, 2003) approximate quantification of volatile compounds was estimated by the integration of peaks on the total ion chromatogram using Xcalibur software (Vienna, VA).

Statistical Analysis

All analyses were carried out in triplicate and the result expressed as mean standard deviation

RESULTS

The result of the antibacterial activity of the samples is presented in Table 1.The formulations had a higher inhibitory activity against *S. aureus* with the highest activity of 16.33mm zone of inhibition. However, there was no significant difference ($p>0.005$) between activity of the formulation from all locations. There was significant difference ($p<0.05$) between the antibacterial activity of the formulations on the other isolates tested as compared with *S. aureus*. *E. coli* was the least susceptible (11.67mm) to the samples although there was no significant difference ($p>0.005$) between the activities on *S. typhi, S. flexnerii* and *B. subtilis*.

The results obtained by GC–MS analysis of the formulations is presented in Tables 1. The formulation had 17 volatile organic compounds. The sample was predominated by chlorobenzene (16.36%), P-xylene (13.77%), 1-methoxy-2-propyl acetate (11.49%) and toluene (11.39%) as the major compounds and methylligrocerate (0.16%), 3,5-Di-tert-butylphenol (0.37%), decane (0.45%), dimethylglutarate (0.59%) as the minor components

Table 1: Antibacterial activity of "Komi da Ruwanka" decoctions sold in Katsina

	Location		
Bacteria species	**KAT1**	**KAT2**	**KAT3**
B. subtilis	$12.33^{b} \pm 0.88$	$12.33^{b} \pm 0.33$	$12.33^{b} \pm 0.33$
S. aureus	$15.67^{a} \pm 0.67$	$15.00^{a} \pm 0.58$	$16.33^{a} \pm 0.33$
S. flexnerii	$13.33^{b} \pm 0.67$	$13.00^{b} \pm 0.58$	$12.67^{b} \pm 0.33$
S. typhi	$12.33^{b} \pm 0.88$	$11.67^{b} \pm 0.33$	$12.00^{b} \pm 0.58$
E. coli	$11.67^{b} \pm 0.44$	$11.67^{b} \pm 0.44$	$11.83^{b} \pm 0.17$

^{a,b,c} Means in a column with the same superscripts are not significantly different (P>0.05)Values are mean ± standard error of three replications

Key :

KAT1= Samples from first location, KAT2 = Samples from second location, KAT3 = Samples from third location

Table 2: Result of GCMS analysis of a traditional formulation also called komi da ruwanka

RT $^{-1}$	**Compound**	**Area normalized (%)**
3.16	Toluene	0.77
4.38	Butylacetate	28.08
4.82	Unknown	6.76
5.60	Unknown	16.96
6.34	1, 3-demethylbenzene (3-xylene)	1.82
6.52	Unknown	6.13
7.43	Uknown	4.13
7.90	2-pinene	3.53
8.46	Unknown	2.02
9.15	2(10)- pinene (is, 5 5) (-)	3.19
10.33	1, 4-cineol (isocineole)	3.98
10.84	Eucalyptol (Cucalyptol)	5.37
12.50	2-butoxyethyl acetate	3.67
12.80	2-carene	2.41
14.09	l-camphor	2.39
15.46	P-cymen-8-01	1.25
15.70	Salicyclic acid, methyl ester	2.12
15.79	P-menth-l-en-8-01 (terpineol)	1.78
22.07	1, 4-methanoazulene	0.48
27.75	Diisobuty phthalate	0.34
27.80	N-hexadecanoic acid (palmitic acid)	0.23
28.76	Methylcis, cis, 9, 12-octudecadienoatees	1.32
29.06	Unknown	1.24

Key: RT^{-1}= Retention time

DISCUSSION

The result of antimicrobial activity of the formulations against the isolates used in this study indicates that *S. aureus* was the most responsive to the formulations. This could be attributed to the fact that all the organisms tested were clinical isolates, which in general have been found to be more resistant to antibacterial agents than non clinical isolates (Lancing *et al.*,1999). This result does not correspond to that of Muhammad, (2008) who reported that the formulation was most active on *S. paratyphi* and *S. typhi*. This implies that the formulation is suitable for infections caused by *S. aureus* thereby confirming the claims of it's producers that the formulation is effective for the treatment of skin infections.

The result of the Gas chromatography-Mass spectrometry analysis revealed the presence of compounds such as salicyalic acid,2-carene, eucalyptol and camphor. Samy, (2000) reported that the presence of compounds such as eucalyptus oil and camphor in plant extracts are responsible for their antimicrobial activity against a wide range of microorganisms. Eucalyptus oil has therapeutic, antimicrobial and biopesticide properties. It is used internally and externally as an expectorant, and to treat infections and fevers. It is also used topically to treat sore muscles and rheumatism (Challand, 2005).

However, this does not correspond with the report of Muhammmad, (2008) who stated that only compounds of benzene and its derivatives were detected in the same formulation. Camphor is readily absorbed through the skin and produces a feeling of cooling similar to that of menthol, and acts as a slightly local anesthetic and antimicrobial substance. There are anti-itch gels and cooling gels with camphor as the active ingredient (Hirota and Hiroi, 1967). Camphor is an active ingredient (along with menthol) in vapor-steam products, such as Vicks VapoRub. A recent publication in Pediatrics suggests the topical application of VapoRub to improve in the symptoms of colds and sleep quality when compared to a control (Lawrence, 2005).

The presence of benzene compounds in the formulation is worthy of concern since exposure to high levels of benzene in air for short periods can cause eye and throat irritation while exposure to higher levels can cause dizziness. Irreversible damage to inner ear and hearing has been observed in animals exposed to relatively low concentrations of ethylbenzene for several days to weeks (Evans, 2001). It has also been shown to cause kidney damage in animals exposed to a relatively low concentration of it for several months. The effect of exposure to other substances detected in the formulation such as P-xylene and acetate include inflammation of the mucous membranes, eczema of the skin and dermatitis (Garriot *et al.*, 1988). Prolonged breathing of toluene vapor is harmful and should be avoided. Tolulene is added to gasoline, used to produce benzene, and used as a solvent. Exposure to toluene may occur from breathing ambient or indoor air. The central nervous system (CNS) is the primary target organ for toluene toxicity in both humans and animals for acute (short-term) and chronic (long-term) exposures. CNS dysfunction and narcosis have been frequently observed in humans acutely exposed to toluene by inhalation; symptoms include fatigue, sleepiness, headaches, and nausea. CNS depression has been reported to occur in chronic abusers exposed to high levels of toluene. Chronic inhalation exposure of humans to toluene also causes irritation of the upper respiratory tract and eyes, sore throat, dizziness, and headache. Human studies have reported developmental effects, such as CNS dysfunction, attention deficits, and minor craniofacial and limb anomalies, in the children of pregnant women exposed to toluene or mixed solvents by inhalation. Reproductive effects, including an association between exposure to toluene and an increased incidence of spontaneous abortions, have also been noted (Streicher *et al.*, 1981).

Conclusion

The analysis of the formulation showed antibacterial activity against all the isolates tested particularly *S. aureus*. So also the results of the GC-MS analysis showed that it contains many substances that are antimicrobial which confirms claims of the herbal vendors that the formulation is effective in treatment of some microbial infections.

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