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Absence of *in-vitro* antimicrobial activity of some antimicrobial herbal preparations in the Ghanaian market

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Abstract
Herbal preparations are formulated into various dosage forms that are used in treatment of diseases including infections. In Ghana, many herbal mixtures are advertised and sold for treatment of microbial infections. The current study sought to investigate the purported antimicrobial activities of four herbal mixtures in the Ghanaian market, using the Agar Diffusion method.
The samples investigated did not exhibit any antimicrobial activity against both Gram-positive and Gram-negative bacteria, and *Candida albicans* used in the study. Most of the plants constituting the components of the herbal mixtures assessed, however, have been documented to have antimicrobial activities against the microbes used in the study.
The inability of the samples to show antimicrobial activity may be attributed to low concentrations of the active compounds in the mixture. Water is the vehicle mostly used for extraction in such mixtures, and it is possible that larger volumes of the vehicle vis-à-vis the plant material resulted in low concentrations of the active compounds. It is also possible that water is not the solvent of choice for such preparations.
The study has shown that some of the common herbal preparations sold in Ghana for management of infections such as Candidiasis do not have the claimed activity *in-vitro*.

Keywords: Herbal mixture, antimicrobial, Ghana, market

1. Introduction
Herbal medicines have been the mainstay of many communities throughout history when it comes to primary healthcare in many developing countries. In the local settings the practice of using herbal medicines evolved from generations that existed long ago. Such knowledge is passed on from one generation to another, mainly through oral history [1]. It is only in, relatively, recent times that documentation of some plants or herbs used in treatment of diseases have become available. Most of the activities concerning herbal medicines are shrouded in secrecy; and it is believed that many rich cultural practices including knowledge in herbal medicines (ethnomedicine) are being lost [2], as some indigenous people rich in such knowledge die without passing it on.
Herbal preparations are formulated into different dosage forms, and are administered for treatment of various ailments. In Ghana, the commonest dosage forms are those for oral administration – syrups, suspension, mixtures, and capsules. Occasionally, some are also administered through the rectal route, either as enema or suppository, and others are used topically. Over the few past years, many herbal preparations have flooded the Ghanaian market for treatment of many diseases. Amongst the commonest diseases or conditions which these herbal preparations are claimed to handle are asthma, infertility in both males and females, infections of bacterial, viral, fungal, protozoal and parasitic origin. Most studies on plants and herbal medicines have looked at the pharmacological activities of plants claimed to have activity against particular diseases. For example if a plant is used in folklore medicine to treat bacterial infection, then a scientific study may look at the *in-vitro* antibacterial activity of the plant. When such a plant is used to formulate an herbal mixture for treatment of bacterial infections, the therapeutic activity of the mixture is rarely investigated; it is assumed that once it is coming from a known potent plant the activity is assured.
In a previous study, plants constituting common antimalarial herbal mixtures sold in Ghana were compiled [3]. In the current one, however, the antibacterial and antifungal activities of four (4) common herbal preparations available in the Ghanaian market and claimed to have good anti-candidal and antibacterial activities have been investigated. These products together contain eleven different plant materials belonging to eleven different plant families. The plants

(and families) constituting the herbal preparations are: *Afzelia africana* (Fabaceae), *Alchornea cordifolia* (Euphorbiaceae), *Alstonia congensis* (Apocynaceae), *Amaranthus spinosus* (Amaranthaceae), *Citrus aurantifolia* (Rutaceae), *Eleucine indica* (Poaceae), *Sida acuta* (Malvaceae), *Spathodea campanulata* (Bignonaceae), *Vernonia amygdalina* (Asteraceae), *Vismia guineensis* (Guttiferae) and *Xylopia aethiopica* (Annonaceae). Each of the mixtures investigated contained at least two of the mentioned plants.

Almost all the mentioned plants are known from literature and ethno-pharmacological studies to have antimicrobial activities and are used in folklore medicine for management of various infections. For example *A. africana* exhibited antibacterial activity against several bacterial species in a study [4]. Similarly, *A. cordifolia* and *A. spinosus* have all shown good antibacterial activities against both Gram-negative and – positive bacterial species [5, 6]. Indigenous people use some of these plants for treatment of diseases such as malaria, and other infections such as gonorrhoea, syphilis, candidiasis. Many products have been formulated, on commercial basis, from the mentioned plants and other plants for treatment of diseases caused by microorganisms. The aim of the current study therefore is to investigate the antimicrobial potential of some local herbal preparations on the Ghanaian market purported to have antimicrobial activity.

2. Materials and Methods

2.1 Sources of Materials

The herbal mixtures used in the study were purchased from local Pharmacies in Kumasi, the Ashanti Regional Capital of Ghana. They were coded HM1, HM2, HM3 and HM4. Two batches of each sample with different Batch Numbers were used in the study. These samples were maintained in their original package and kept in a cool dry place in the Laboratory until required for use.

2.2 Micro-organisms Used

The micro-organisms used in the study comprised Type and Clinical Isolates of Gram-positive bacteria and Gram-negative bacteria. The Type Cultures were *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCTC 10073), *Klebsiella pneumoniae* (ATCC 70063); the clinical isolates included *Pseudomonas aeruginosa, Salmonella typhi, Neisseria gonorrhoea* and the yeast-like fungus, *Candida albicans* (which was the only fungal species used).

2.3 Antimicrobial Assay

The agar well diffusion method, used to determine the zones of inhibition described by an antimicrobial agent, was used in the assay. Nutrient agar plates were seeded with 100 µl of an overnight broth suspension of bacteria or fungi containing approximately 10^6 cfu/ml of the microorganism. Different plate was prepared for each microorganism. Cork borer number three with a diameter of 6 mm was used to make 'wells' on the agar in the plate. Six wells were made on each plate; four of them were labelled with the codes of the herbal samples (HM1, HM2, HM3 and HM4), one was labelled with the standard antibiotic, amoxicillin (AMX), used as control and the last one, water for injection (WFI). In the case of the plate seeded with *Candida albicans*, clotrimazole (CLT) was used in place of amoxicillin. One hundred microlitres (100 µl) each of the herbal mixtures and the standard drug was introduced into the corresponding labelled well in the agar. Whilst the control drugs (amoxicillin and clotrimazole) was each prepared with water for injection to a concentration of 100 µg/ml, the mixtures were used in their original state, none was diluted. The plates were allowed to be on the laboratory bench for about an hour (h) before incubating at 37 ºC for 24 h. The antimicrobial activities of the samples were evaluated by taking measurements of the diameters of the zones of inhibition around each well.

3. Results and Discussion

In formulating herbal mixtures, irrespective of the pharmacological use, several methods are available. The commonest is decoction, where the plant material is boiled and the liquid (extract) decanted for use. There is also infusion. Here boiling (hot) water is poured on the plant material, and the liquid portion of the mixture taken. Preparation of tea for consumption is a typical example of infusion. In some other methods the plant material is soaked in ethanol, and the ethanol extract is drunk from time to time.

The vehicle (solvent) used in extraction of a medicinal plant material is very important. The ability of the solvent to extract the medicinal components (constituents) of the plant is paramount in its choice. Therapeutic activity of plants depend on the active constituent(s). Not all the active constituents may be extracted by say water; it is therefore important that the appropriate solvent must be selected for the extraction.

In the study, it was observed that none of the herbal mixtures, was able to inhibit the growth of any of the microorganisms, namely, *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae Neisseria gonorrhoea* and *Candida albicans* used. The herbal mixtures coded HM1, HM2, HM3 and HM4 are all claimed to have the ability to cure diseases such as gonorrhoea, syphilis and candidiasis caused by microorganisms; and these indications together with others are written on the labels of the products (herbal mixtures). The products were tested at their original concentrations, after an initial 1:1 and 1:2 dilution in water for injection had failed to inhibit the growth of any of the microorganisms. When the first batch of samples could not arrest the growth of the microbes after triplicate investigations, a second batch with different batch numbers for each sample was acquired. The second batch also could not inhibit the growth of any of the microbes in triplicate experiments. The summarised results are as found in Table 1.

Table 1: Antimicrobial activities of some herbal mixtures (at original concentrations) and standard antimicrobial agents as controls (at 100 µg/ml).

Microorganism	Average Zones of Inhibition (mm)					
	Herbal Mixtures				Controls	
	HM1	HM2	HM3	HM4	AMX	CLT
Staphylococcus aureus	*	*	*	*	23	Nt
Bacillus subtilis	*	*	*	*	24	Nt
Klebsiella pneumoniae	*	*	*	*	23	Nt
Salmonella typhi	*	*	*	*	21	Nt
Pseudomonas aeruginosa	*	*	*	*	24	Nt
Neisseria gonorrhoea	*	*	*	*	21	Nt
Candida albicans	*	*	*	*	Nt	24

Key: HM1 – Herbal Preparation 1; HM2 – Herbal Preparation 2; HM3 – Herbal Preparation 3; HM4 – Herbal Preparation 4; AMX – Amoxicillin; CLT – Clotrimazole. * - No observable zone of inhibition; Nt – Not tested

Each of the herbal mixtures contained at least two of the following plants; *Afzelia africana, Alchornea cordifolia, Alstonia congensis, Amaranthus spinosus, Citrus aurantifolia, Eleucine indica, Sida acuta, Spathodea campanulata, Vernonia amygdalina, Vismia guine ensis* and *Xylopia aethiopica*. Most of these plants have been reported to have antimicrobial activities against most of the bacteria and the fungus used in the study. For example ethyl acetate extract of the leaves of *A. cordifolia* [7], exhibited good antimicrobial activity against *P. aeruginosa, S. aureus and C. albicans*; the methanol extract of the plant has also shown antibacterial activity against multi-drug resistant bacteria [8]. Similarly, methanolic leaf extract of *V. amygdalina* showed a broad spectrum activity against *S. aureus, K. pneumonia, P. aeruginosa* and *C. albicans* [9, 10]. Further, *S. acuta* [11]; *A. africana* [4]; *X. aethiopica* [12]; *V. guineensis* [13] and *S. campanulata* [14] has each exhibited antimicrobial activity against many species of bacteria and fungi, including some of the species used in the study. However, in a sharp contradiction of activities, none of the herbal preparations made from combinations of these plants showed any ability to inhibit the growth of the microbes.

The absence of antimicrobial activities of the herbal mixtures purported to have the ability to inhibit or arrest the growth of bacteria and fungi may be attributed to the solvent used in the extraction. Water, though may be considered as a universal solvent, may not be the solvent of choice for many organic compounds. Most studies where pharmacological activities of aqueous extracts and that of other organic solvents such as ethanol or acetone are compared show that the non-aqueous extracts mostly give better activity [15].

In the local settings (homes) some of these plants are prepared as decoctions for use in management of infections, and the users seem to obtain the required therapeutic activity. Here the proportion of water to plant material may be ideal. However, in preparation of the same product on commercial basis a relatively larger volume of water and less amount of plant material may be used. Such preparations may have less amount of active constituents, than those prepared in households, and this can result in failure of therapeutic activity when used; noting that the antibacterial activities of most plant extracts are concentration dependent [16].

Additionally, other factors such as age of plant (material), time of collection [17], location of the plant and storage conditions of the plant material may all affect the amount of active constituents which is responsible for the therapeutic activity.

The active compound(s) accountable for any pharmacological activity of a plant may reside in a particular morphological part of the plant. For some it is the stem bark, others the leaves or the roots; the fruits, flowers and seeds may also be used. The morphological parts of the plants used in preparation of the herbal mixtures were not indicated on the label, neither the amount (portions) of each sample present. More often than not, the activities of herbal medicine preparations (type and part of the plant, treatment of the plant material and the preparation method) are mostly shrouded in secrecy. This is, perhaps, to enable them (the practitioners) protect the knowledge in herbal medicine from getting into public domain as no one person can lay claim for discovery of plant activity or patent a plant for its therapeutic activity. Secondly, medicinal plants are available in the wild, in fact very few people cultivate them, so letting out the knowledge in herbal medicine would mean that anybody can have access to prepare and use. From the foregoing, it may be possible that a morphological part of the plant other than the one with the intended pharmacological activity was used in some of the herbal preparations.

The current study, having shown that some of the herbal preparations sold for treatment of infections in Ghana have failed to exhibit such an activity, behoves on the regulatory bodies of the country to carry out post-registration surveillance on herbal products. Additionally, manufacturers of herbal preparations, especially those for commercial use must receive adequate training on collection, treatment, preparation methods and standardisation of the products.

4. Conclusion

Four common herbal preparations available in the Ghanaian market purported to have antimicrobial activities did not show any such activity in an *in-vitro* study. The preparations contain at least two of the following plants: *Afzelia africana, Alchornea cordifolia, Alstonia congensis, Amaranthus spinosus, Citrus aurantifolia, Eleucine indica, Sida acuta, Spathodea campanulata, Vernonia amygdalina, Vismia guineensis* and *Xylopia aethiopica*. The lack of activity may be attributed to low concentrations of the active compounds of the constituting plants in the mixture; as at least one plant in each of the mixtures has exhibited potent antimicrobial activity in other studies.

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