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Comparison of *in vitro* Growth Inhibitory Activities of Aqueous Extracts of Selected Individual Plants with the Multi-Component Concoction.

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Abstract

Reports have shown an increased occurrence of bacterial infections due to the rapid development of multi-drug resistant strains of bacteria. In this regard, lots of efforts are being made to discover new drugs. Medicinal plants have played an important role in drug discovery. Multiple-plant herbal concoctions (multi-component herbal concoctions) are used as remedies in many cultures around the world. This is due to the advantages of synergism and low dosage of active principles compared with single-plant herbal remedies. An aqueous concoction obtained from a combination of leaves of *Psidium. guajava*, *Acacia albidia* and *Ficus exasperata* is used for the treatment of bacterial infections amongst many tribes in northern Ghana. In the present study, aqueous extracts of *P. guajava*, *A. albidia* and *F. exasperata* were screened separately for phytochemicals and their bacterial growth inhibitory activity evaluated in vitro using the agar diffusion method with *Escherichia coli* and *Salmonella typhi* as test organisms. The result was compared with that of an aqueous concoction obtained from a combination of the three plants (multi-component concoction) to determine any indication of synergism or additive effect. Ciprofloxacin was used as control. Phytochemical screening of the extracts revealed the presence of phytochemicals with reported antimicrobial activities. All the extracts exhibited antibacterial activity with the multi-component extract demonstrating a superior antibacterial property against the test organisms at concentrations ranging between 1.0-5.0 mg/ml. The antibacterial activity of the multi-component concoction was statistically not significantly different from that of Ciporfloxacin (P = 0.87). There was no indication of synergism or additive effect in the multi-component concoction.

Keywords: Growth inhibitory activity, Multi-component herbal formulation, Herb-herb interaction, Synergy, Antagonism, Phytochemical screening

INTRODUCTION

Herbs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health. In spite of the significant advances made in modern medicine in recent times, plants still play an important role in primary health care and drug discovery. Over the past decades, interest in drugs derived from higher plants, especially the phytotherapeutic ones, has increased impressively[1].

Herbal medicine has become a popular form of healthcare. According to the World Health Organization (WHO), about 65-80% of the world's population which lives in developing countries depends essentially on plants for primary health care[2]. Even though several differences exist between herbal and conventional pharmaceutical treatments, herbal medicine can be tested for efficacy using conventional trial methodology. Several specific herbal extracts have demonstrated to be efficacious for specific conditions. Though the public is often misled to believe that all natural treatments are inherently safe, some herbal medicines have severally demonstrated some level of toxicity.

In many societies around the world, multi-component herbal concoctions have been used in the treatment of a wide range of diseases. In multi-component herbal therapy, extracts from either a combination of different parts of the same plant or a combination of parts of different plants are used in the formulation of concoctions. The concept of multi-component herbal therapy may be beneficial when the individual plants or plant parts in the concoction possess different efficacies that provide additive or synergetic effects. It may also reduce the required doses of the individual components compared with mono-component herbal therapy and limit side effects.

The components in a multi-component herbal mixture that are deemed beneficial can be ranked into four types; the imperial herb which is the chief herb and the main ingredient of the formulation, the ministerial herb which augments and promotes the actions of the imperial herb, the assistant herb which reduces the side effect being caused by the chief herb and the servant herb which harmonizes or coordinates the action of the other herbs[3].

On the other hand, some individual herbs in a multi-component concoction may have a negative effect on the overall efficacy of the multi-component herbal formulation due to masking and other chemical or physical interactions. In effect, herb-herb interactions in a multi-component herbal concoction may also result in antagonism. This area of study has attracted little attention in the scientific world hence the need to conduct appropriate studies on effects of possible herb-herb interactions on efficacy.

Choru liri (C.L) is an aqueous multi-component herbal concoction used in the treatment of bacterial infections in northern Ghana. It is the aqueous extract obtained by

combining the leaves of *Psidium guajava*, bark of *Ficus exasperata* and bark of *Acacia* albidia in hot water.

The Guava plant, *Psidium guajava* is of the family *Myrtaceae*. Aqueous leave extracts of *P. guajava* are used in folk medicine as a remedy for diarrhoea and, as well as the bark, for their supposed antimicrobial properties and as an astringent[4]. The leaves have also been reported for their use in traditional treatment of diabetes in parts of northern Ghana. In Trinidad, a tea from the leaves of *P. Guajava* is used in the treatment of diarrhoea, dysentery and fever[5].

Ficus exasperata is a large evergreen banyan tree of the *Moraceae*. It is best known for its beautiful buttress roots, which are also known for damaging municipal footpaths. The stem bark of *F. exasperata* has been reported for its use in the treatment of diarrhoea[6]. It has also been reported for its antibacterial properties[7,8].

Acacia albida is a species of *Faidherbia* native to Africa and the Middle East, formerly widely included in the genus *Acacia*. Common names for it include Apple-ring Acacia, Ana Tree and Winter Thorn[9]. It is of the family *Mimosoideae*. Phytochemical studies reveals that plants in this family contain tannins[10]. It is also used to treat fevers by the Masai people of Kenya as well as for diarrhoea in Tanganyika[11]. Previous studies has also demonstrated that the plant possess anti-pyretic, anti-inflammatory, and anti-diarrhoea[10] properties.

There are several convergences in the use of *P. guajava*, *F. exasperata* and *A. albida* in traditional medical practices. This justifies the continued use of a concoction prepared from these plants in the treatment of bacterial infections by people of northern Ghana.

The present study seeks to evaluate in vitro the anti-bacterial activities of leave extract of *P. guajava*, stem bark extract of *F. exasperata* and stem bark extract of *A. albida* using *Escherichia coli* and *Salmonella typhi* as test organisms.

The anti-bacterial activity of the multi-component concoction prepared from these three plants will also be evaluated and compared with those of the individual plants. The result may provide an insight as to whether the extract obtained from a combination the three plants would produce a synergistic, additive or antagonistic effect on the test organisms.

MATERIALS AND METHODS

SOURCE OF MATERIALS

Plant material

Psidium guajava (guava) leaves, *Acacia albidia* stem bark and *Ficus exasperata* stem bark, were collected at Gongnia, a suburb of Navrongo in the Kassena Nankana District of the Upper East Region of Ghana. The plant materials were taken to the Department of Applied Biology of the University for Development Studies where they identified by Dr. Isaac Sackey, a Botanist.

Reagents

All reagents used were of analytical grade unless otherwise stated and were purchased from Sigma-Aldrich.

Bacteria strains

Escherichia coli and *Salmonella typhi* bacteria were cultured at the University For Development-DANIDA Microbiology laboratory.

METHODS

Preparation of extracts

Individual plant extracts

Adequate quantities of individual plant samples were air dried for 48 hours and milled to powder. About 500g of each powdered sample was weighed separately in a 1000 ml beaker and about 500 ml distilled water added. This was allowed to boil for about 30 minutes. The crude extract obtained was concentrated using rotary evaporator and then freeze dried.

Multi-component concoction

Air dried samples of leaves of *P. guajava*, stem bark of *F. exasperata* and stem bark of *A. albida* were milled into powder separately and then 200g of each powder was taken and mixed with the others to give a combined mass of about 600g. The combined mass was then transferred into beaker and approximately 600 ml distilled water was added. This was allowed to boil for about 30 minutes. The crude extract obtained was concentrated using rotary evaporator and then freeze dried.

Phytochemical analysis

About 5g of each freeze dried sample of the crude extract was dissolved to about 100 ml and portions analyzed for phytochemicals using standard methods with little modifications.

Test for alkaloids

About 2 ml of the plant extract is stirred with few drops of 1% HCl on a steam bath. The solution obtained was filtered and 1ml of the filtrate was treated with 1 drop of Mayer's reagent. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract.

Test for saponins

About 2 ml of the plant extract was introduced into a test tube and stirred on a water bath for about 5 minutes, the mixture was then vigorously shaken for 2 minutes. Formation of honey comb fronts indicates the presence of saponins.

Test for cardiac glycosides

About 2 ml of conc. H_2SO_4 was transferred into a test tube. Approximately 1 ml of aqueous crude extract were mixed with 1ml of glacial acetic acid (CH_3COOH) containing 1 drop of $FeCl_3$. The resulting mixture above carefully added to the 1ml H_2SO_4 so that the H_2SO_4 is underneath the mixture. The appearance of a brown ring was indicative of the presence of cardiac glycosides.

Test for steroids

About 2 ml of the plant extract was dissolved in 2ml chloroform and an equal volume of conc. H_2SO_4 was added by the sides of the test tube. If the upper layer turns red and the sulphuric layer shows yellow with green fluorescence, then there is indication of the presence of steroids.

Test for tannins

About 2 ml of the plant extract was boiled for 5 minutes. Two drops of 5% $FeCl_3$ was then added.

Formation of greenish precipitate indicates the presence of tannins.

Test for amino acids

About 2 ml of the extract is treated with Ninhydrin reagent (n-butanol). Appearance of purple colour indicates the presence of amino acids.

Test for anthraquinones

About 2 ml of the extract was hydrolysed with dilute conc. H_2SO_4 and extracted with benzene. About 1 ml of dilute aqueous NH_3 was then added. Rose pink colouration suggests a positive test.

Test for flavanoids

Few drops of dilute NaOH was added to 2 ml of the extract. The appearance of an intense yellow colour which turns colourless on addition of a few drops of dilute HCl indicates the presence of flavanoids.

Test for terpenoids

About 2 ml of the plant extract was mixed with 2 drops $CHCl_3$ in a test tube. About 3 drops of conc. H_2SO_4 was then carefully added to the mixture to form a layer. An interface with a reddish brown colouration is formed if terpenoids are present.

Test for phlobatannins

About 2 ml of the plant extract was boiled with 2% HCl. The formation of a red precipitate shows the presence of phlobatannins.

Test for reducing sugars

To 2 ml of the solution of the extract in a test tube, about 0.5ml each of Fehling's solutions A and B was added. The mixture was shaken and heated in a water bath for 10 minutes. Formation of brick-red precipitate indicates the presence of reducing sugar.

Test for soluble starch

About 2 ml portions of extract was boiled with 1ml of 5% KOH, cooled and acidified with H_2SO_4. Yellow colouration was taken as the presence of soluble starch.

Preparation of Mueller-Hinton agar

About 19 g of Mueller-Hinton was weighed into a 500ml conical flask about 500 ml of distilled H_2O was then added and agitated. The content was heated to boil on a hot plate with magnetic stirrer until the powder completely dissolved in the water and the pH adjusted to 7.3±0.1. The media is poured into a media bottle well corked and autoclave at 121°C for 15 minutes. The media was well labelled and stored in the refrigerator until use.

Antimicrobial susceptibility test

The spreading method of Cruickshank et al[12], and the well agar diffusion method of Garrod et al[13], were used. The test microorganisms were inoculated on nutrient agar plates and spread uniformly using a sterile glass spreader. Wells of 5 mm in diameter were made on the nutrient agar using a sterile cork borer. The cut agar disks were carefully removed by the use of forceps sterilized by flaming. Different concentrations of the freeze extracts were prepared by dissolving various masses in 20% dimethyl sulphoxide (DMSO). To each well was introduced different concentrations (1.0, 2.0, 3.0, 4.0, 5.0 mg/ml) of plant extracts. Control experiments were set up using Ciprofloxacin and DMSO as positive and negative controls respectively. The plates were allowed to stand for one hour at room temperature for diffusion of the substances to proceed before the growth of microorganisms commenced. The plates were made in triplicate and were incubated at 37°C for 24 h. Diameters of zones of inhibition in the triplicate plates were measured by calculating the difference between cork borer (5mm) and the diameters of inhibition[14,15,16]. The zones of inhibition were then recorded.

Statistical analysis

Data collected in the study are expressed as the mean ± standard error of mean (S.E.M.) and statistical analysis was carried out by using unpaired t-test.

RESULTS AND DISCUSSION

Phytochemical screening of plant extracts

Preliminary phytochemical screening of crude aqueous extracts of the various individual plants and the concoction revealed varying composition of phytochemicals.

P. guajava leave extract showed the presence of alkaloids, saponins, cardiac glycosides, steroids, tannins, flavanoids, terpenoids, phlobatanins and reducing sugars (Table 1). Most of these phytochemicals have been widely reported for their antimicrobial activity. Amino acids, anthraquinones and soluble starch were however absent in the *P. Guajava* leaves.

Table 1: Phytochemical analysis of extracts

Phytochemical constituent	*P. guajava*	*F. exasperata*	*A. albidia*	Multi-component concoction
Alkaloids	++	+	+	+
Saponins	++	+	+++	+++
Cardiac glycosides	++	+	+	+++
Steroids	+	++	-	++
Tanins	++	+	++	++
Amino acids	-	-	-	+
Anthraquinones	-	+	+	-
Flavonoids	++	+	+	++
Terpenoids	++	+	++	++
Phlobatanins	+	+	-	++
Reducing sugars	+	+	++	++
Soluble starch	+	-	+	+

- = absent + = present ++ = moderately abundant +++ = highly abundant

F. exasperata stem bark extract showed the presence of alkaloids, saponins, cardiac glycosides, steroids, tannins, flavanoids, terpenoids, phlobatanins, amino acids and anthraquinones. Soluble starch was absent in this extract (Table 1).

Phytochemical screening of aqueous *A. albidia* stem bark extract revealed the presence of all the phytochemicals tested with the exception of steroids, amino acids and phlobatanins (Table 1).

The concoction prepared by combining the three plants showed the presence of all the phytochemicals tested with the exception of anthraquinones. Most of the phytochemicals which tested positive in the concoction appeared in a relatively high abundance (Table 1).

Results from the phytochemical screening are consistent with the reported antibacterial potentials of the individual plants and of the concoction. This justifies the use of these extracts in traditional medicine. Further to this, the observed high abundance of some of the tested phytochemicals in the concoction is an indication of a possible increase in their concentrations resulting from combining the three plants (Table 1).

However, the absence of anthraquinones in the concoction may be due to masking or chemical disintegration resulting from herb-herb interaction.

Antimicrobial activity

The extracts showed varying antimicrobial activities at different concentrations. The concoction showed the greatest antimicrobial activity against both bacteria at concentrations ranging from 1mg/ml to 5mg/ml. (Zone of inhibition ranged from 1.00±0.50mm to 18.00±0.30mm against *E. coli* and from 0.00±0.00mm to 18.00±0.05 against *S. thyphi*.) (Table 2). The extract from stem bark of *F. exasperata* showed the least activity on both bacteria within the same concentration range. (The zone of inhibition ranged from 0.00±0.00mm to 7.70±0.05mm against *E. coli* and 0.00±0.00mm to 8.33±0.03) (Table 2). This result may be due to the relatively low abundance of the phytochemicals tested for in *F. exasperata* stem bark extract (Table 1).

Although the antimicrobial activity resulting from the combination of the three plants (as in the concoction) does not seem to suggest any additive or synergetic effect, the activity of the concoction was much greater than those of the individual plant extracts and was statistically not significantly different from that of the control (P = 0.87).

Antibacterial activity demonstrated by the concoction may suggest overlaps in the type of phytochemicals present in the individual plants. Thus, the individual plants may contain identical compounds with similar mode of actions, and combining the plants in a single remedy will merely increase their concentrations. The increased concentrations of the phytochemicals in the multi-component concoction may not have translated into an additive or synergistic effect.

CONCLUSION

The individual aqueous extracts of leaves of *P. guajava*, stem bark of *A. albidia* and stem bark of *F. exaperata* were screened for phytochemicals and their antibacterial activities evaluated in vitro using *E. coli* and *S. typhi* as test organisms. These extracts demonstrated antibacterial properties and this was supported by the type of phytochemicals present in each extract. Aqueous multi-component concoction obtained from the combination of the three plants demonstrated a superior antibacterial activity comparable to that of Ciprofloxacin. This result justifies the use of the concoction in the treatment of bacterial infections in the northern part of Ghana. The antibacterial activity of the multi-component was not a demonstration of synergistic or additive effect.

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Table 2. Antibacterial activity of aqueous extracts and control on test organisms

Conc. Of extracts mg/ml	Mean Diameter of Zone of Inhibition (mm±SEM)									
	P. guajava		*A. albidia*		*F. exasperata*		*Multi-component concoction*		Ciprofloxacin	
	E. coli	*S. typhi*	*E. coli*	*S. typhi*	*E. coli*	*S. typhi*	*E. coli*	*S. typhi*	*E. coli*	*S. typhi*
1.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	1.00 ±0.03	0.00 ±0.00	3.00 ±0.00	2.00 ±0.03
2.00	0.00 ±0.00	2.00 ±0.10	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	4.00 ±0.10	2.00 ±0.00	6.00 ±0.10	8.00 ±0.30
3.00	9.70 ±0.03	5.30 ±0.05	7.00 ±0.00	5.00 ±0.00	4.70 ±0.00	3.30 ±0.00	10.00 ±0.20	10.00 ±0.00	9.00 ±0.20	12.00 ±0.10
4.00	11.30 ±0.00	10.70 ±0.10	9.50 ±0.03	6.30 ±0.05	5.33 ±0.40	4.70 ±0.30	14.00 ±0.00	12.00 ±0.10	12.50 ±0.03	18.00 ±0.00
5.00	14.70 ±0.00	12.67 ±0.00	15.50 ±0.09	9.20 ±0.50	7.70 ±0.05	8.33 ±0.03	18.00 ±0.30	18.00 ±0.05	20.00 ±0.00	22.00 ±0.00

Data collected were subjected to statistical analysis using t-test

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