



ANTIMICROBIAL EXAMINATIONS OF *CYMBOPOGON CITRATUS* AND *ADIATUM CAPILLUS-VENERIS* USED IN GHANAIAN FOLKLORE MEDICINE

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ABSTRACT

The antimicrobial activity and Minimal Inhibitory Concentration (MIC) of the extracts of *Cymbopogon citratus* and *Adiatum capillus-veneris* were evaluated against four bacteria (*Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and a fungus (*Candida albicans*). These plants are used in Ghanaian folk medicine to treat infections of microbial origin. The antibacterial and antifungal activities were tested using agar diffusion technique. The ethanol extracts of the two plants showed appreciable antimicrobial and antifungal activity against *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Candida albicans* with MIC of 0.78mg/ml and 12.5mg/ml for *C. citratus* and *A. capillus-veneris* respectively. However, the aqueous extract of *Cymbopogon citratus* showed no activity against the tested organs but that of *Adiatum capillus-Veneris* had activity against *Proteus mirabilis* and *Klebsiella pneumonia*. All the plants show different kinds of phytochemicals. The phytochemical investigation revealed the presence of sugars, flavanoids, triterpenoids, and steroids for *A. capillus-veneris* and flavonoids, anthraquinones, alkaloids, saponins, phenols and steroids for *C.citratus*. Statistical analysis using student t-test showed no statistical difference between MICs of the two plants and chloramphenicol.

Key words: Antimicrobial activity, Minimal Inhibitory concentration, *Cymbopogon citratus*, *Adiatum capillus-veneris*.

INTRODUCTION

In developing countries like Ghana, low income earners and rural dwellers use traditional medicine which is mostly extracts from plants for the treatment of common infectious diseases. These plants are ingested as decoctions, teas and juice preparations for treatment. They are also made into a poultice and applied directly on the infected wounds or burns (Gonzalez, 1980).

According to the World Health Organization report in 2002, earlier investigations revealed that

four billion people, representing 80% of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous peoples' tradition, a common element in ayurvedic, homeopathic, naturopathic, traditional oriental, and Native American Indian medicine. It is also estimated that, of the 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by

native cultures (WHO, 1993). Current research by major pharmaceutical companies on plant materials gathered from the rain forest region and other potential places focus on medicinal value of these plants (Asaolu, 2003)

Many investigators have demonstrated the antimicrobial activity of the constituents of some higher plants. Quite a number of the chemical compounds of plant origin have shown to possess antimicrobial activities (Tagoe et al, 2011a).

Most microorganisms have become resistant to the first line antibiotics such as Chloramphenicol, tetracycline, and even combine drugs like penicillin-streptomycin. Similarly, orthodox drugs have adverse effect on those who use them (WHO, 2006, Tagoe et al, 2011b).

One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents (Shah, 2005). Traditional healers claim that some medicinal plants such as *Bixa* spp. and *Bidens* spp. are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non severe cases of infectious diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant (Fabricant and Farnsworth, 2001).

In the light of this, the present study looks at two species used in folk medicine; lemon grass (*Cymbopogon citratus*) and maiden hair fern (*Adiantum capillus-veneris*) and their combination to determine their antimicrobial activity. This is in pursuance of the efforts to search for drugs from plants to be use as an antimicrobial drug for the treatment of the test microbes (*Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Candida albicans*).

C. citratus, commonly known as lemon grass oil, is a tropical plant from Southeast Asia, which is often sold in stem form. Its leaves are use to make tea, which can relieve stomach and gut problems. It can

also act as an anti-depressant and as a mood enhancer. It thrives in warmer temperate regions and not hardy to frost. In Brazilian folk medicine, it is believed to have anxiolytic, hypnotic and anti-convulsant properties, but at least one study has found no effect in humans. It is abundant in the Philippines, and said to have 65-85% citral composition. *C. citratus* contains active ingredients like myrcene, an antibacterial and pain reliever, citronella and geraniol (Blanco *et al.*, 2009).

Citral is one of the most important biological active substances isolated from *C. citratus* which has been found to aid digestion, relieve spasms, muscle-cramps, rheumatism and headache (Russo, 1992). A tea made from the leave of *C. citratus* has been used to treat fever, cold, cough, stomach upset and has diuretic properties and can help in urinating difficulties and water retention (Stehmann, & Brandaw, 1995). Extracts of both the leaves and stalks of *C. citratus* are also used as herbal medicine to treat nervous condition and inflammation (Ramirez *et al.*, 1988). Further investigations have revealed the use of lemon grass in some countries in Africa, Latin American and in Asian cuisine.

A. capillus-veneris, the black maiden hair fern, is a species of fern in the genus *Adiantum* with a sub cosmopolitan distribution, native to western and southern Europe, Africa, North and Central America. It has long held a place in herbal medicine systems worldwide. In France, the fronds and rhizomes, once made into syrup called "Sirop de Capillaire," was a favorite medicine for upper respiratory problems such as coughs and excessive mucus. The plant is also use widely throughout the world for dandruff, hair loss, and menstrual difficulties (Husson *et al.*, 1986).

In Brazil, the frond and leaf are employed to treat hair loss, coughs, bronchitis, laryngitis and throat dryness, as well as to improve appetite and digestion, stimulate renal function, regulate menstruation, and facilitate childbirth. Also, in Peruvian herbal medicine, the fronds and rhizomes are used for hair loss, gallstones, hepatic calculi, hydrophobia, asthma, coughs, catarrh, and to regulate menstruation. In India, the entire plant is used for its

cooling effects, for treating diabetes, colds, bronchial disease, and for its menstrual promoting properties. The plant is externally used for boils, eczema, and wounds treatment (Neef *et al.*, 1995).

The fronds are use as a garnish on sweet dishes. When dried they are also use to make tea. Syrup has been made from the plant for a refreshing summer drink. The fern is simmered in water for several hours and the liquid made into thick syrup with sugar and orange juice for a refreshing drink, which is used in the treatment of coughs, throat afflictions and bronchitis (Facciola, 1990).

The maidenhair fern has a long history of medicinal used and was the main ingredient of a popular cough syrup called 'Capillaire', which remained in use until the nineteenth century. The plant has little use in modern herbalism. The fresh or dried leafy fronds are antidandruff, antitussive, astringent, demulcent, depurative, emetic, weakly emmenagogue, emollient, weakly expectorant, febrifuge, galactogogue, laxative, pectoral, refrigerant, stimulant, sudorific and tonic. It is also used as detoxicant in alcoholism and to expel worms from the body (Foster& Duke, 1990). Externally, it is used as a poultice on snake bites and bee stings among others (Foster& Duke, 1990, Moerman, 1998). In Nepal, a paste made from the fronds is applied to the forehead and chest to relieve headaches and chest pains respectively. The plant is best use fresh, though it can also be harvested in the summer and dried for later use (Manandhar, 2002). Chemical analysis of maidenhair fern has revealed an array of compounds including triterpenes, flavonoids, phenylpropanoids, and carotenoids. But, despite of its ancient use, there has not been any specific research to isolate and test its chemicals components for biological activities (Neef *et al.*, 1995).

Scientists in Iraq demonstrated that maidenhair fern had antimicrobial properties. A methanol extract of the aerial parts reported to have *in vitro* antimicrobial actions against *Bacillus*, *E. coli*, *Staphylococcus*, *Proteus*, *Pseudomonas*, and *Candida* with little toxicity (Mahmoud *et al.*, 1989).

In animal studies, however, maiden hair ferns found to have an anti fertility effect. In the 1980s, two

separate investigations conducted in India revealed that a petroleum ether extract of the plant had an anti-implantation effect in rats, preventing conception (Neef *et al.*, 1995).

Despite the maiden hair fern ancient history of use for respiratory disorders, no clinical research has been carried out to validate these traditional uses. Also, in spite of the lack of scientific research done on maidenhair fern, herbalists and healthcare practitioners throughout the world continue to use the plant based on its traditional uses for respiratory disorders and hair loss, and to regulate menstruation.

Evidently, there are not sufficient scientific studies that confirm the antimicrobial properties of these plants. This study looks into the *in vitro* antimicrobial activity of these plants against six pathogenic microorganisms that cause the most common cases of infectious diseases in poverished communities in Ghana.

MATERIALS AND METHODS

1. Collections and preparation of materials

1.1 Collections of materials

Laboratory grade of Ethanol from British Drug Houses was obtained from the Centre for Scientific Research into Plant Medicine Microbiology Laboratory, Akuapim-Mampong in Eastern Region of Ghana. Muller Hinton agar and broth were equally obtained from the centre for microbial analysis.

The plants were collected locally in Cape Coast, Ghana, identified and confirmed by the plant curator of the herbarium at Department of Environmental studies, School of Biological Sciences, University of Cape Coast, Ghana where a voucher specimen of the plants are deposited. The plants were sun dried and powdered.

The organisms used were clinical isolates of *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Candida albicans* all from the the Centre for Scientific Research into Plant Medicine Microbiology Laboratory, Akuapim-Mampong in Eastern Region of Ghana.

1.2 Preparation of plant extracts

The plant extracts were prepared using the modified method of Alade & Irobi (1993). Briefly, two 100 g portions of the dried powdered plant were soaked separately in 500 ml of distilled water and ethanol (98 %) for 72 h. Then, each mixture was filtered with sterile Whitman's No. 1 filter paper. The filtrates were stored in the refrigerator at 4°C until required. The ethanol filtrate was concentrated under vacuum at 40°C to obtain the dry crude extract. The aqueous filtrate was freeze-dried to reconstitute the extract into powdered form. About 1g of sample obtained was added to 5ml of the sterilized distilled water and kept at 4°C until required.

2. CHEMICAL ANALYSIS

2.1 Phytochemical investigation

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as tannins (phenazone; iron complex; formaldehyde and modified iron complex test), cardiac glycosides (Keller-Killiani and Kedde tests), and alkaloids (Mayer's; Drangendorff's; Wagner's and 1% picric acid reagents).

For flavonoids, Shimoda's test was adopted (15 mg of dry extract was dissolved in 1 ml of ethanol, concentrated HCl, and magnesium turnings were added). Anthocyanins were identified by adding 1 ml of boiling water, 0.5 ml of 37 % HCl to 10 mg of dry extract. The solution was heated at 100°C, cooled and added 0.4 ml of amylic alcohol.

The test for phenolic compounds was carried out by dissolving 10 mg of dry extract in 1 ml of 1 % ferric chloride solution. Quinones were identified by extracting 10 ml of the aqueous extract with dichloromethane, evaporating the organic phase, and adding 5 ml of ethanol, 1 ml of hydrogen peroxide 5 % and 1 ml of sulfuric acid 50 % respectively. The solution was heated, cooled, extracted with benzene and 1 ml of ammonia solution added.

For triterpenoids and steroids, 0.5 ml of acetic anhydride and 1 droplet of 37 % sulfuric acid solution were added to 0.5 ml of the organic phase.

2.2 Antimicrobial activity

The antimicrobial activities of the extracts on test organisms were determined by agar diffusion bioassay as described by Cheesbrough (2006).

3.0 STATISTICAL ANALYSIS

All values are expressed as means \pm standard deviation (Table 2). The MIC data for each microorganism were analyzed using one-way analysis of variance (ANOVA) and the differences among group means were analyzed using the Dunnett's multiple comparisons test. P value < 0.05 was considered as significant. The data obtained was analyzed using One Sample-Test.

RESULTS AND DISCUSSION

All the plants showed antimicrobial activity in regards to at least three microorganisms tested (Table 1). The ethanol extracts of *A. capillus-veneris* was the most active against the microorganisms studied. In some cases, the two extracts of the same plant had antimicrobial activity against the same microorganism. For instance, the two extracts of *A. capillus-veneris* were active against *P. mirabilis* and *K. pneumonia*. This possibly means that the compound responsible for the antimicrobial activity was present in each extract at a different concentration.

All the plants exhibited different kinds of secondary metabolites. Ethanol extracts for the two plants displayed higher microbial inhibitory activity than the aqueous extracts. This result indicates that water decoction is a bad method to extract the active ingredients found in this species. This finding is correlated with the medicinal preparations that use rum and liquor to extract the active plant components.

S. aureus, and *K. pneumonia* were the most susceptible bacteria to all plant extracts even though the aqueous extract of *C. citratus* did not exhibit any inhibitory effect on any of the test microorganisms. On the contrary, *P. aureginosa* and *C. albicans* were

the most resistant microorganisms. Apart from the ethanol extract of *A. capillus-veneris* and the combined ethanol extracts of the two plants that exhibited higher inhibitory activity against *K. pneumonia* (15.00±0.58), than the positive control, none of the extracts was more active against any of the test microorganisms than the positive control (chloramphenicol). Likewise, no extract was active against *P. aeruginosa*. Only the ethanol extract of *C. citratus* and the combined ethanol extracts of both plants were active against *C. albicans*. The presence of saponins, alkaloids, phenols, steroids, flavonoids

and anthraquinones in the extracts could be responsible for the level of antimicrobial activity of the extracts. *A. capillus-veneris* and *C. citratus* showed no activity against *P. aureginosa* and *C. albican*. Also, aqueous extracts of both plants showed no activity against *S. aureus*. This agrees with literature (Asaolu, 2009). Meanwhile, the ethanol extracts of both plants showed some level varying degree of activity with that of *A. capillus-veneris* having higher degree of activity (Table 1.0). However, investigation conducted by Mamoud and his team compared favourably with our result (Mamoud et al., 1989).

Table 1.0: Antimicrobial activity of 20% (w/v) Ethanol and Aqueous Extract of *Cymbopogon citratus* and *Adiantum capillus-veneris* using the agar diffusion method.

Medium	Extracts	Diameter of Zones of Inhibition (mm)				
		<i>Proteus mirabilis</i>	<i>P. aureginosa</i>	<i>S. aureus</i>	<i>K. Pneumonia</i>	<i>C. albican</i>
Aqueous	<i>C. citratus</i>	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	<i>A. Capillus-veneris</i>	11.33±0.33	0.00±0.00	0.00±0.00	12.33±0.88	0.00±0.00
	<i>C. citratus + A. capillus-veneris</i>	10.00±0.00	0.00±0.00	0.00±0.00	10.67±0.33	0.00±0.00
Ethanol	<i>C. citratus</i>	0.00±0.00	0.00±0.00	7.00±0.00	0.00±0.00	10.00±0.00
	<i>A. capillus-veneris</i>	7.00±0.00	0.00±0.00	14.67±0.00	15.00±0.58	0.00±0.00
	<i>C. citratus+A. Capillus-veneris</i>	6.33±0.33	0.00±0.00	16.00±1.00	15.00±0.58	5.00±0.00
Control	Chloramphenicol (50µg/ml)	16.00±2.08	25.33±2.33	21.33±1.85	14.67±1.33	22.67±0.33

Table 2.0: Minimum Inhibition Concentration (MIC) recorded for the Ethanol and Aqueous extract of *Cymbopogon citratus* and *Adiantum capillus-veneris* against the test organisms.

Medium	Test organism	Minimum Inhibition Concentration (mg/ml)		
		<i>C. citratus</i>	<i>A. capillus-veneris</i>	combine
Ethanol	<i>Staphylococcus aureus</i>	0.78	50	0.78
	<i>Klebsiella pneumonia</i>	-	12.5	0.78
	<i>Proteus mirabilis</i>	-	12.5	0.78
	<i>Candida albican</i>	12.5	-	32
Aqueous	<i>Klebsiella pneumonia</i>	-	50	50
	<i>Proteus mirabilis</i>	-	50	50

From the phytochemical screening, *A. capillus-veneris* exhibited the least variety of secondary metabolites. However, it showed the larger spectrum of antimicrobial activity against the pathogens studied. Sugars, flavonoids, triterpenoids and steroids found in this plant might account for this. Table 2 shows that *C. citratus* and *A. capillus-veneris* presented the same MICs against *C.*

albicans, *P. mirabilis* and *K. pneumonia* (12.5 mg/ml). However, *C. citratus* manifested a better MIC against *S. aureus* (0.78) similar to the combined ethanol extracts of the two plants against *S. aureus*, *K. pneumonia* and *P. mirabilis*. However, there was no statistical difference between MICs of the two plants and chloramphenicol.

CONCLUSION

The extracts showed varying degrees of antimicrobial activity on the microorganisms tested. The plants were generally less effective than the traditional antibiotics to combat the pathogenic microorganisms studied except *Adiantum capillus-veneris* on *K. pneumonia*. However, it is worthy to note the synergistic ability of the combined ethanol extracts of the two plants to inhibit the growth of *S. aureus*. The chance to find antimicrobial activity was more apparent in ethanol than water extracts of both plants. *Adiantum capillus-veneris* presented the

broadest spectrum of antimicrobial activity. This plant could be a source of new antibiotic compounds. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity.

This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. However, none of the plants are recommended in the treatment of infections produced by *C. albicans* and *P. aeruginosa*.

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