



Original Research Article

Antimicrobial effects of *Chromolaena odorata* on some human pathogens

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ABSTRACT

Keywords

Antimicrobial effects;
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Minimum and Inhibitory Concentration.

Antimicrobial effects of *Chromolaena odorata* on some known human pathogens (*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*) were carried out. Different solvents such as ethanol and cold water were used for the leaf extraction. The minimum inhibitory concentration (MIC) of ethanol extract against *Staphylococcus aureus* and *Escherichia coli* were 0.25mg/ml and 0.125mg/ml respectively. The MIC of cold water (aqueous) extract on *Staphylococcus aureus* and *E. coli* showed no activity. For the fungi *Candida albicans*, the ethanolic extract of leaf showed MIC at 0.25 while aqueous (cold water) extract showed MIC at 0.5 dilutions. This indicates that ethanolic extraction is more effective than cold water. This justifies the therapeutic use of *Chromolaena odorata*. Also further investigations can combine the plant extracts with another medicinal plant for synergic effects.

Introduction

It is well known that nature holds many secrets, however, since ancient times man has learned some of its secrets. Among the most useful ones, were those related to how medicinal plants could heal and ameliorate diseases. Indigenous plants widely used for folk medicinal purposes are numerous and diverse. In Bangladesh about 500 plant

species have been identified as medicinal plants because of their therapeutic properties (Ghani, 2000). A medicinal use of plant is the oldest healthcare known to mankind.

Although modern medicinal science has been developed to a great extent, many

rural people still depend on plant products and herbal remedies for treating their ailments. Being naturally gifted by a suitable tropical climate and fertile soil. Nigeria posses a rich flora of tropical plants. About 5000 species of phanerograms and pteridophytes grow in its forests, jungles, wastelands and roadside as indogenous, naturalized and cultivated plants. Out of them, more than a thousand have their medicinal and therapeutic use (Yusuf *et al*,1994). In addition to possessing various other medicinal properties, 257of these medicinal plants have been identified as efficacious remedies for diarrhea disease and 47 for diabetes. A large number of plants in different location around the world have been extracted and semi-purified to investigate individually,their antimicrobial activity (Dranghon, 2004). For instance, *Garcinia biflavonone* has been found to be active against a wide variety of micro-organisms such as *Salmonella typhi*, *Escheria coli* and *Staphyloccocus aureus*(Iwu,1993). It is also used in treatment of liver disorder and throat infection. The root of *Nauclea latifolia* has antibacterial activity against gram positive. It is most effective against *Corynebacterium diphtheria*, *Streptobacillus species*, *Streptococcus species*, *Pseudomonas aeruginosa* and *Salmonella species* (Louis, 1999).

Furthermore, some extracts of green pepper, garlic and onions have been noticed to inhibit the growth of *Shigella dysenterialae* and *Salmonella typhi*. *Pterocarpus santalinoide* are used as vegetables in food preparation and the leaves are as folder for feeding live stock. *Senna alata* has been identified as a medicinal plant used in the cure of many ailments and diseases in many parts of the world and the leaves are taken orally as an

effective laxative and are used in caese of constipation.

However, fresh juice squeezed out from the leaves of *Chromolaena odorata* is used to stop bleeding. The decoction of the leaves and stems are reported to be effective against the treatment of skin disease like *Propionibacterium acnes* (Chakraborty *et al.*, 2010).

It is also used for healing wounds, effective against KI strain of Plasmodium which causes malaria, possess anti-gonorrheal, anti-inflammatory, anthelmintic, analgesic, antioxidant and antifungal activities. *Chromolaena odorata* is also an ornamental flower.

The aim of this study was to know the antimicrobial effects of the water and ethanol extracts of *Chromolaena odorata* leaves on some pathogens, such as *Staphyloccocus aureus*, *Eschericha coli* and *Candida albicans*.

Materials and Methods

Collection of Samples

The medicinal plant used in this study was the leaves *Chromolaena odorata* (siam weed). They were obtained from Umudike in Ikwuano L.G.A., Abia State, Nigeria. The samples were immediately transported to the laboratory for use. The test organisms were obtained from the Federal Medical Centre, Umuahia, Abia State.

Preparation of the leaves

The plant materials were collected and washed to reduce microbial load to a large extent. They were further air dried to remove water on the leave surfaces. The leaves were further dried in the laboratory

oven at 60°C for two days. After sufficiently dried, a warring industrial blender was used to crush the leaves to powder and then weighed.

Ethanolic Extract Preparation

100g each of pulverized powdered leaves of plant materials were weighed using an electronic weighing balance and weighed sample were soaked separately in two clean 250ml conical flasks containing 200mls of ethanol each(one of the conical flasks to be used for antibacterial assay and other for antifungal assay).The mixture was vigorously stirred with a stirrer. After 72hrs with interval stirring, the mixture was filtered using a clean filter papre(whatman filter paper) into a clean beaker and the filtrate was concentrated to dryness by evaporation using a steam bath at 90⁰C for 48hrs.The standard extracts obtained were then stored in the refrigerator at 4⁰ C. For the antifungal assay, the mixture was left for three weeks before filtration and the filtrate was concentrated by evaporation using steam bath at 90°C for 48hrs.The standard extract obtained was then stored at 4°C as stated by Naidoo *et al*(2011).

Aqueous (cold water) Extract Preparation

100g of the pulverized powdered leaves of plant materials was weighed and soaked separately into two 500ml conical flasks each containing 400mls of distilled water. The mixture was vigorously stirred with stirrer. After 72hrs interval stirring, the mixture was filtered using a clean filter cloth into a clean beaker and the filtrate was concentrated to dryness by evaporation using a steam bath at 90⁰ C. The standard extract obtained was stored in refrigerator at 4°C.For the antifungal

assay, the mixture was left for three weeks before filtration as stated by Naidoo *et al* (2011). The extract were stored for use. The yield was recovered as percentage of the quantity of the initial plant materials 100g used (Ekwenye and Nnaemeka, 2005).

Sterilization

All equipment were washed with detergent and rinsed thoroughly with water as described by Cheesbrough, 2000). They were sterilized. All the media used were according to manufacturer's specification.

Preparation of Disc

Disc of diameter 6mm were perforated from the whatman filter paper using a perforator. The discs were sterilized using a bijou bottle at 160⁰ C for 2hrs in a hot air oven. It was brought out and allowed to cool before further use according to Robert *et al* (2011).

Preparation of Antibiotic Disc

250mg and 200mg each of chloramphenicol and clotrimazole were separately dissolved in 2mls of water each to give 500mg/ml and 400mg/ml each.1 ml of the various stock solutions was diluted in 1ml of water and 1 ml of this stock was added to 10 paper discs in glass petri dish and allowed to dry in the oven 3hrs at 40⁰ C, so that the drug would stick to the discs(Robert *et al* .,2011).

Disc Preparation for Ethanolic Extract

1 gram of ethanol extract of leaves was mixed with 2mls of water. The mixture was properly done in a test tube and 1ml of the mixture was poured into a glass petri dish containing 10 paper discs. The

disc was put in the oven to dry so as to allow the plant extract stick to the paper disc for further use (Robert *et al.*, 2011).

Disc Preparation of Aqueous Extract (cold water)

1 gram of leaf extract was mixed with 2mls of water. The mixture was thoroughly done in a test tube. 1ml of the mixture was poured into a glass petri dish containing 10 paper discs. The petri dish containing the disc was put in the oven for drying (Robert *et al.*, 2011).

Media Preparation

All the materials used were sterilized after being washed with detergent and rinsed severally with distilled water. The media used for culturing the organism were nutrient agar for *Staphylococcus aureus* and *Escherichia coli* (bacterial) and Sabouraud Dextrose Agar (SDA) for *Candida albicans*.

Nutrient agar was prepared by dissolving 6.72g of agar powder in 240mls of distilled water and autoclaved at 121⁰C for 15 mins and allowed to cool to body temperature. The media was dispensed into petri dishes, it was flamed to remove air bubbles. Sabouraud Dextrose agar media was prepared by dissolving 7.8g of agar powder in 120mls of distilled. It was autoclaved for 15mins at 121⁰C and allowed to cool to body temperature before dispensing into petri dishes. It was flamed was flamed to remove air bubbles accprding to Cheesbrough (2000).

Susceptibility Test Using Plant Extract

SDA and nutrient agar plated were inoculated with respective test organisms using syringe and needle followed by

spreading using glass spreader for each test organism. Plates were in triplicate for each test organism in aqueous and ethanol extract. The plates were allowed to dry for 15 mins in an incubator. The dried water and ethanol discs as mentioned above were transferred using flamed but cooled forceps into the surface of the inoculated agar plates. They were sufficiently spaced to prevent the resulting zone of inhibition from overlapping. The plates were incubated at 37⁰C for 18-24hrs to observe the zone of growth inhibition produced by the extract (Naidoo *et al.*, 2011).

Susceptibility Test Using Antibiotics

Antboitic sensitivity test was carried out on all isolates using Kirby Bauer's paper discs diffusion techniques. Chloramphenicol and clotrimazole were used. After the inoculation of test organisms into various agar plates. The plates were allowed to dry in an incubator for 15mins. The antibiotic discs were placed on the agar using sterile forceps. Each disc was placed far from each other to prevent their zones of inhibition from overlapping. The plate with the antibiotic disc were then incubated at 37⁰C for 2hours to observe the zone of inhibition produced by the antibiotics (Naidoo *et al.*, 2011).

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of the extract was determined by incorporating constant volume (1ml of each dilutions of the extract unto the perforated on a nutrient agar plates as described in the antimicrobial susceptibility testing section. 1g of each extract was dissolved in 1 ml of sterile water to obtain 1000mg/ml. This 1000mg/ml concentration was then doubly

diluted in sterile distilled water to obtain concentration of 1/2,1/4,1/8,1/16, 1/32,1/64, 1/128 and 1/256mg/ml. MIC was also determined for the antibiotic used. The stock of each of the antibiotic used was doubly diluted in sterile water to obtain concentration of 1/2,1/4,1/8,1/16, 1/32,1/64,1/128,1/256mg/ml.

Results and Discussion

After evaporation, percentage of ethanolic yield of plant extract was 3.8%, while percentage yield of aqueous extract was 3.5%. In this current study, the gram positive and gram negative bacteria (*Staphylococcus aureus* and *E.coli*) used were both inhibited by the ethanol extracts of *Chromolaena odorata*. *E.coli* had the highest zone of inhibition among the two bacteria. On the other hand, aqueous extracts had no effect on both *S.aureus* and *E.coli*. However, chloramphenicol which was used as the positive control had the highest power of inhibiting the micro-organisms.

Furthermore, in the case of the fungi *Candida albicans*, the ethanol extract inhibited the growth of the fungi while aqueous extract showed very minute inhibition. The control drug clotrimazole used as the positive control had the highest power of inhibition. The MIC of ethanol extract of *Chromolaena odorata* on *Staphylococcus aureus* was 0.25(1/4) and on *E.coli* was 0.125(1/8) dilutions and on *C.albicans* was 0.125 while cold water, the MIC was 0.5(1/2) dilution.

The study showed no antimicrobial activity against *Staphylococcus aureus* and *E.coli* using aqueous extraction of *Chromolaena odorata*. This probably

indicates that cold water could not extract the bio-active ingredients in the plant (Naidoo *et al.*, 2011).

However, the ethanolic extraction of *Chromolaena odorata* against *Staphylococcus aureus* and *E.coli* were very effective. The phytochemical analysis on the aqueous extract of the plants revealed the presence of steroids, tannins, saponins, alkaloids and flavonoids. Most of these compounds have been shown to act against most micro-organisms. This study also showed that the ethanol extract of *Chromolaena odorata* was effective against *Candida albicans* but cannot be compared on its effects on *Staphylococcus aureus* and *E.coli*. Aqueous extraction had a minimal effect on *Candida albicans*. Above all, the control drug chloramphenicol for bacteria and the control drug clotrimazole for fungi retained the highest antibacterial and antifungal effect.

This study highlighted the antimicrobial effects of *Chromolaena odorata* on some known pathogens. Some antibiotics have been obsolete because of the problem of drug resistance. Thus improvement of health using herbs as raw materials should be reconsidered.

Chromolaena odorata is a known invasive weed in Nigeria and readily spreads with ease inhabiting any available space. The ability of *Chromolaena odorata* exhibiting antimicrobial activities in the current research work indicates a potential for alternative use of the weed as raw materials for the production of medicine that can be used in diseases caused by *Staphylococcus spp*, *Escherichia coli* and *Candida albicans*.

Table.1 Gram of extract with respect to solvent

Plant	Solvents	Yield(g)	Yield(%)
<i>C.odorata</i>	Ethanol	3.8	3.8
<i>C.odorata</i>	Aqueous(cold)	3.5	3.5

The percentage yield was calculated against 100g product of the plant material subjected to each extraction method.

Table.2 Antimicrobial activity of ethanolic and aqueous plant extract

Organism	<i>C.odorata</i>	<i>C.odorata</i>
	Ethanol	Aqueous
<i>E.coli</i>	7.0+/-0.1	Na
<i>Staphylococcus aureus</i>	6.0+/-0.1	Na
<i>Candida albicans</i>	4.0+/-0.1	2.0+/-0.1

Note diameter:diameter(mm)

Na-No activity

Table.3 Minimum inhibitory concentration of ethanol plant extract on test micro-organism

Ethanol Extract	1(1)	½(0.5)	¼(0.25)	1/8(0.0125)	1/16(0.0625)	1/32(0.03125)	MIC
<i>S.aureus</i>	6	4	2	-	-	-	0.25
<i>E.coli</i>	7	6	4	3	-	-	0.125
<i>C.albicans</i>	4	2	1	-	-	-	0.25

Table.4 Minimum inhibitory concentration of aqueous plant extract on test micro-organism

Ethanol Extract	1(1)	½(0.5)	¼(0.25)	1/8(0.0125)	1/16(0.0625)	1/32(0.03125)	MIC
<i>S.aureus</i>				-	-	-	
<i>E.coli</i>					-	-	
<i>C.albicans</i>	2	1		-	-	-	0.5

Note:(-)-No zone of inhibition

Table.5 Diameter (mm) zone of inhibition produced by antibiotic

Pathogen	Antiboitic
	Chloramphenicol
<i>Staphylococcus aureus</i>	7+/-0.1
<i>Escherichia coli</i>	8+/-0.1
	Clotrimazole
<i>Candida albicans</i>	7+/-0.1

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