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Full Length Research Paper

Phytochemical and antibacterial properties of *Combretum mucronatum* (Schumach) leaf extract

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The cold extraction method was used to obtain the methanol extract of the leaf of *Combretum mucronatum.* The extract was analyzed for antibacterial activities, using some pathogenic bacteria namely: *Klebsiella pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Bacillus cereus, Salmonella typhi* and *Bacillus subtilis*. The antibacterial bioassay was carried out *in-vitro* and it revealed that the methanol leaf extract inhibited the growth of the tested organisms at a concentration of 25.0 mg/ml except *K. pneumoniae* and *S. pyogenes* which were resistant. The extract exhibited the highest inhibitory potential on *S. aureus* with a zone of inhibition value of 35.0 mm at a concentration of 25.0 mg/ml. This was followed by *E. coli and P. aeruginosa* which were inhibited with zones of inhibition values 30.0 mm and 25.0 mm respectively. *B. cereus* was the least inhibited with a zone of inhibition of 16.0 mm. Result of the phytochemical screening tests revealed that the extract contains saponin, tannins, anthraquinone and cardiac glycoside. The rate at which the extract was able to kill the test organisms showed that the organisms decreased with increased time of exposure to the extract. *P. aeruginosa* decreased to zero at the 24th hour. The minimum inhibitory concentration (MIC) of the leaf extract ranged from 25.0 to 3.12 mg/ml. The result of the antibiotic sensitivity test compared well with the commercial antibiotics.

Key words: Antibacterial, zone of inhibition, phytochemical screening, extracts, rate of killing.

INTRODUCTION

Man and plants

The generality of the life of man is 100% dependent on the plants in our environment (Selby, 1998). This means that the diversity of plant species in the world is so useful for our adaptation to the environment and existence.

A medicinal plant is defined by the World Health Organization (WHO) consultative group as any plant in which one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (World Health Organization, 2003).

The medicinal plants with high therapeutic and prophylaxis value are mostly from the plants that are herbaceous and the majority of the world's population uses such herb products as a primary source of medicine.

Traditional medicine

The term traditional medicine (Indigenous medicine or folk medicine) describes medical knowledge systems, which developed over centuries within various societies before the era of modern medicine. WHO defines traditional medicine as the health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being (World Health Organization, 2003).

The use of traditional medicine is now spreading in most countries of the world with over 80% of the primary

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health care needs based on the extract from these medicinal plants. The WHO also notes, though, that "inappropriate use of traditional medicines or practices can have negative or dangerous effects" and that "further research is needed to ascertain the efficacy and safety" of several of the practices and medicinal plants used by traditional medicine systems (World Health Organization, 2003).

Modern medicine and herbal medicine

Modern medicine has provided many breakthrough treatments for serious diseases. Some conditions, however, have eluded the healing grasp of contemporary western medicine, which emphasizes rigorous scientific investigation of therapies. In addition, rising costs of some treatments have placed modern healthcare beyond the reach of many. The drugs that routinely fill pharmacy shelves of post-industrialized nations remain inaccessible to the majority of the people in the world. At the same time, more antibiotic pathogens are emerging by the day. Now, populations in many areas of the globe use herbal medicine also called botanical medicine or phytotherapy, as the principal means of healthcare.

Herbal medicine is the use of natural plant substances to treat illness. Based upon hundreds, even thousands of years of experience, herbal medicine provides an alternative to modern medicine, making healthcare more available. In fact, the majority of the world's population uses herb products as a primary source of medicine. While some regulating authorities fear the consequences of unrestricted herbal remedy use, herbal medicine offers a degree of hope to some patients whose disease states do not respond favorably to modern pharmaceuticals. More often, however, herbal remedies are used to treat the common ailments of daily living like indigestion, sleeplessness, or the common cold. Resurgence in interest in herbal medicine has occurred in the United States as medical experts have begun to recognize the potential benefit of many herbal extracts. So popular has herbal medicine become that scientific clinical studies of the effectiveness and proper dosing of some herbal medicines are being investigated.

Herbal medicine recognizes the medicinal value of plants and plant structures such as roots, stems, bark, leaves, and reproductive structures like seeds and flowers. To some, herbal medicine may seem to be on the fringes of medical practice. In reality, herbal medicine has been in existence since prehistoric time and is far more prevalent in some countries than is modern healthcare.

The use of herbs ground into powders, filtered into extracts, mixed into salves, and steeped into teas has provided the very foundation upon which modern medicine is derived (Barney, 1996). Indeed, herbal medicine is the history of modern medicine. Many modern drugs are compounds that are derived from plants whose pharmacological effects on humans had been observed long before their mechanisms of action were known.

A common example is aspirin. Aspirin, or acetylsalicylic acid, is a compound found in the bark of the willow tree belonging to the taxonomic genus Salix. Aspirin, now sold widely without prescription, is an effective analgesic, or pain reliever, and helps to control mild swelling and fever. While aspirin is synthetically produced today, willow bark containing aspirin was used as an herbal remedy long before chemical synthesis techniques were available. Similarly, the modern cardiac drug digitalis is derived from the leaves of the purple foxglove plant, *Digitalis purpurea.* Foxglove was an herbal known to affect the heart long before it was used in modern scientific medicine.

Description of plant

Combretum mucronatum is a usually dark green plant that clings to other plant around for support and exposure to sunlight for its photosynthetic activities. It is widely distributed in West Africa and could be found in the Savanna forest of the region. The plant is mainly found in Western Nigeria especially during the rainy season.

The plant has been used extensively in traditional medicine for the treatment of a variety of diseases. The leaves and roots are used in traditional medicine for treatment of wounds, cough, dysentery, and as anthelmintic, antimicrobials, and antipyretic (Sofowora, 1982). The decoction from the leaves and root is given for the treatment of venereal disease in woman (Wallis, 1967).

This work is intended to assay for the antibacterial and phytochemical properties of the leaf extract of *C. mucronatum* with a view to verifying its traditional use as a medicinal plant and to examine whether its action on the said bacteria are cidal or static.

MATERIALS AND METHODS

Leaves of *Combretum mucronatum,* conical flasks, spatula, forceps, Bunsen burner and tripod stand, stirring rod, beakers, measuring cylinder, test tubes, test tube rack, cotton wool, filter paper, petri dishes, aluminum foil, nutrient agar, sterile water, methanol and nutrient agar.

Collection and preparation of plant leaf

The plant leaves used for this project (*Combretum mucronatum*) were collected on January 13, 2008 from the forest and wild life reserve of Federal University of Technology, Akure, Nigeria, where they were found growing naturally. They were identified at the department of Crop Science and Production of the Federal University of Technology, Akure, Nigeria. A voucher specimen was submitted at the departmental herbarium.

Table 1. Antibacterial activity of the methanol extract of *Combretum mucronatum*.

Organisms	Zone of inhibition(mm)/at the concentration of 25.0 mg/ml
Staphylococcus aureus	35.0
Escherichia coli	30.0
Pseudomonas aeruginosa	25.0
Streptococcus pyogenes	–
Salmonella typhi	18.0
Klebsiella pneumoniae	–
Bacillus subtilis	25.0
Bacillus cereus	16.0
Proteus mirabilis	20.0

Collection and identification of bacterial samples

Bacterial isolates were collected at Don Bosco Catholic Medical Centre, Araromi Street, Akure, Ondo State, Nigeria. The bacterial isolates used for this research work included: *Salmonella typhi, P. mirabilis, E. coli, S. aureus, B. subtilis, B. cereus, Klebsiella pneumoniae, P. aeruginosa* and *S. pyogenes.*

Extraction of leaves

The leaves were air dried for six weeks and crushed into powder. Exactly 600 g of the powder was soaked in methanol for 72 h, after which it was first sieved with a muslin cloth, then filtered using No 1 Whatman filter paper. The filtrate was collected in a beaker and dried *in vacuo.*

Pytochemical screening

The pytochemical screening was done according to the method described in Trease and Evans (1996).

Determination of antibacterial activities of leaf extract

The antibacterial activity of the plant extracts was assayed using agar dilution method described by Olutiola et al. (1991). The concentration of the extract used is 25 mg/ml. The plates were incubated at 37°C for 24 h. Clear zones around the bored holes are indicative of the inhibition of the organisms by the extract.

Determination of minimum inhibitory concentration

Five concentrations (25, 12.5, 6.25, 3.12 and 1.56 mg/ml) of the methanol extract of *C. mucronatum* leaf were assayed, the method of (Trease and Evans, 1996) was used. The plates were incubated for 24 h at 37°C.

Concentration of the crude extract that showed zone of inhibition, below which there was no inhibition was recorded as the minimum inhibitory concentration.

Antibiotics sensitivity test

The disc diffusion method described by (Khan et al., 2002) was used.

Standardization of bacterial samples

A 10 ml of 18 h old broth culture was centrifuged at 2000 rpm for 10 min. It was decanted to retain the residual cells and 10 ml of normal saline was added. It was then centrifuged to wash the cells. The supernatant was decanted and this process was done four times to wash the cells. The washed cells were stored with 15 ml of normal saline.

Determination of the rate of killing of organisms by the extract

A 5 ml of 25 mg/ml of the methanol extract and 5 ml of the standard culture was added together in a sterile test tube. The solution was allowed to stand for 24 h, for interaction between the organism and the extract. At intervals of one hour, 1 ml of the solution was pour plated using nutrient agar. The microbial load was determined, after incubation at 37°C for 24 h.

RESULTS AND DISCUSSION

C. mucronatum leaves are extensively used to treat wounds, cough, dysentery, helminthic infections, bilious, pyretic and generally used as antimicrobials. Results from this work corroborates its use as antimicrobial. Table 1 shows that the methanol extract of *Combretum mucronatum* inhibited all the tesed bacteria except *S. pyogenes* and *Klebsiella pneumonia*. *S. aureus* showed the highest susceptibility with the highest zone of inhibition value of 35.0 mm, hence its traditional use in the treatment of wounds (Wallis, 1967) seems rational. This was followed by *E. coli* and *P. aeruginosa* with zones of inhibition values of 30 and 25mm respectively. Studies have implicated *S. aureus, E. coli*, and *P. aeruginosa* among others as leading causative agents of nosocomial and community infections (Branger et al., 2005; Oteo et al., 2005)

The high susceptibility of these organisms is a clear indication of the effect of the leaf extract as a good treatment for the bacterial infections caused by the trio. This further confirms its traditional use in the treatment of dysentery.

Table 2. Phytochemical groups in dried leaf extracts of *Combretum mucronatum*.

Phytochemical groups	Presence/absence
Saponin	+ve
Tannins	+ve
Phlobatannin	-ve
Alkaloids	-ve
Anthraquinone	+ve
Cardiac glycoside	
Legals Test	+ve
Salkowski Test	+ve
Keller Killian Test	+ve
Liebermans Test	+ve

+ = Present; - = Absent.

Table 3. Minimum inhibitory concentration of the methanol extract of *Combretum mucronatum* against the bacterial isolates.

Organisms	Concentration of methanol extract (mg/ml)
Staphylococcus aureus	3.12
Escherichia coli	3.12
Bacillus cereus	3.12
Proteus mirabilis	6.25
Salmonella typhi	12.5
Bacillus subtilis	3.12
Psedomonas aeruginosa	3.12

Klebsiella pnuemoniae and *S. pyogenes* were not susceptible to the extract. It is however possible that an increase in the concentration of the extract, or its purification may cause the extract to inhibit these two organisms. Again, if the extract from this plant is used in synergy with the extract from another medicinal herb, an inhibition of these two organisms may be effected. Oloke (1997) demonstrated the effectiveness of synergism between tetracycline, bacterim and seed extract of *Aframomum memegueta.*

Antimicrobial activity in plants have been reported to be as a result of bioactive components present in the plants, such as alkaloids, saponins, tannins, anthraquinones, steroids, flavonoids etc (Harbone, 1984; Odugbemi, 2006). Table 2 shows the presence of various bioactive com-ponents in the extracts. These are most likely responsible for broad spectrum inhibitory effect shown on Table 1.

The minimum inhibitory concentration (MIC) tests of the leaf extract on the test organisms as shown on Table 3 indicated that the MIC of the methanol extract of *C. mucronatum* is at 3.12 mg/ml for all the test organisms except *S. typhi* and *P. mirabilis*, which were 12.5 and 6.25 mg/ml respectively. This low MIC values also show that the extract has a strong antibacterial effect on the test organisms.

The effect of the commercial antibiotics on both gram positive and gram negative bacteria compares well with that of the crude extracts used in this study (Table 4). *S. pyogenes* and *K. pnuemoniae* were resistant to all the antibiotics except to gentamycin and tetracycline. This duo also showed resistant to the extract in this study.

The effect of the extract on the test organisms can be considered to be of a broad spectrum action because both gram positive and gram negative bacteria were inhibited. This effect is also seen from the result of the rate of killing of the organisms by the extract (Figures 1 and 2). The number of organisms present at each hour declined till the 24th hour. The extract had a bactericidal effect on *P. aeruginosa* which got totally killed within the 24 h of exposure.

This research was conducted on crude extract; it is believed that if the extract is further purified, stronger inhibitory results will be achieved and the structure of the active phytochemical components can also be determined. Furthermore, an assay of the toxicological analysis will assess its safety and level of tolerance in mammalian body.

Herbal medicine has proven to be of great importance to the treatment of basic human diseases from time

Table 4. Antibiotic sensitivity test on bacterial isolates.

Gram positive organisms	Zones of inhibition (MM)							
	GEN	**PEN**	**STR**	**TET**	**AMP**	**CHL**	**CXC**	**ERY**
Staphylococcus aureus	25.0	_	20.0	13.0	_	13.0	_	26.0
Streptococcus pyogenes	15.0	_	_	15.0	_	_	_	_
Klebsiella pneumoniae	14.0	_	_	8.0	_	_	_	_
Bacillus subtilis	13.0	_	11.0	_	_	8.0	_	_
Bacillus cereus	15.0	_	13.0	_	_	10.0	_	_
Gram negative organisms	**GEN**	**NAL**	**NIT**	**COL**	**STR**	**TET**	**AMP**	**COT**
Escherichia coli	17.0 mm	22.0	15.0	11.0	10.0	_	_	_
Pseudomonas aeruginosa	10.0	_	_	10.0	9.0	_	_	_
Salmonella typhi	14.0	19.0	14.0	10.0	13.0	_	_	_
Proteus mirabilis	12.0	_	_	_	10.0	18.0	_	16.0

GEN-Gentamycin PEN-Penicillin; STR-Streptomycin; TET-Tetracycline; AMP-Ampicillin; ERY- Erythromycin; CHL-Chloramphenicol; NAL-Nalidixic acid; NIT-Nitrofurantoin; (COT-Cotrimazole.

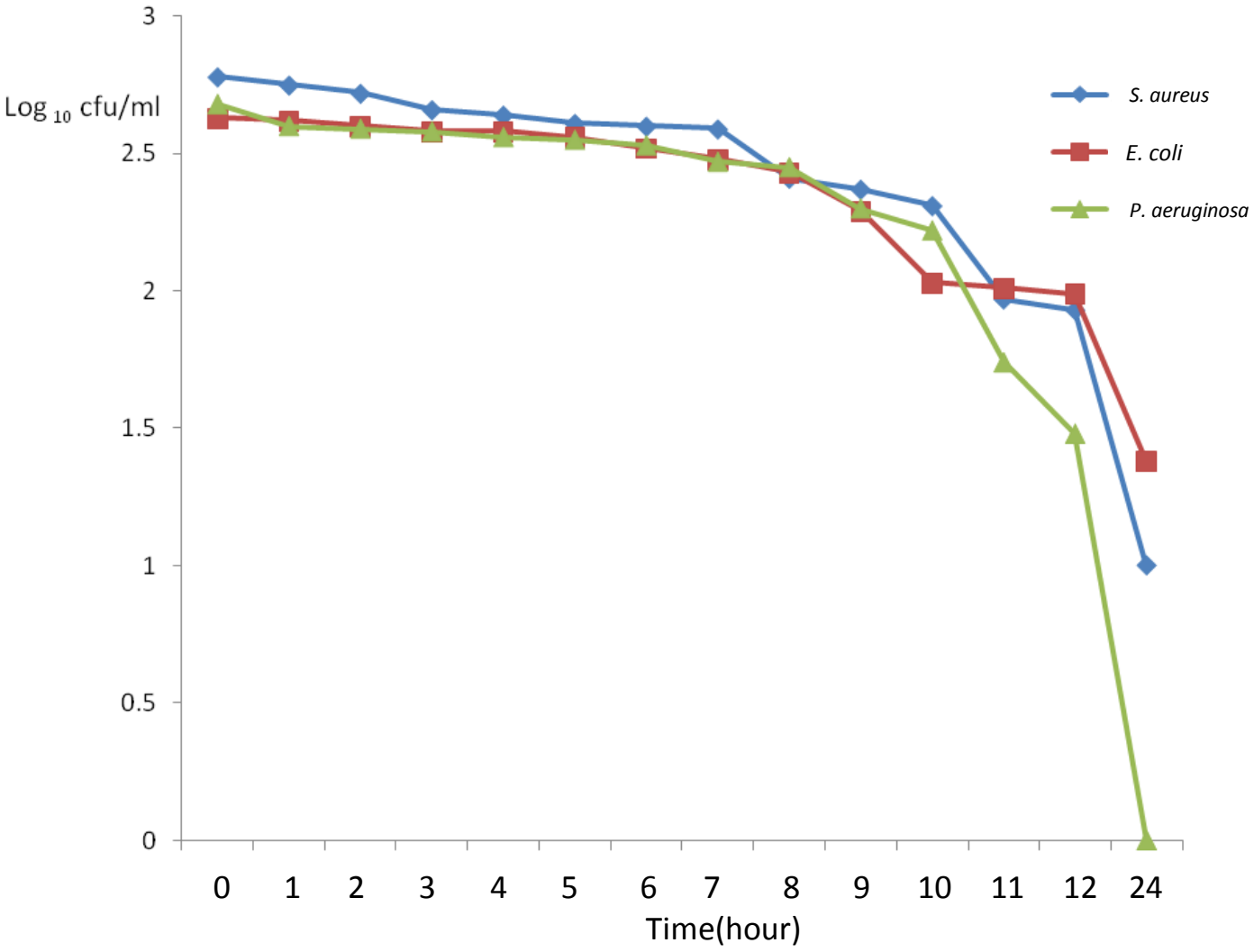

Figure 1. Rate of killng of some bacterial isolates by *Combretum mucronatum* leaf extract.

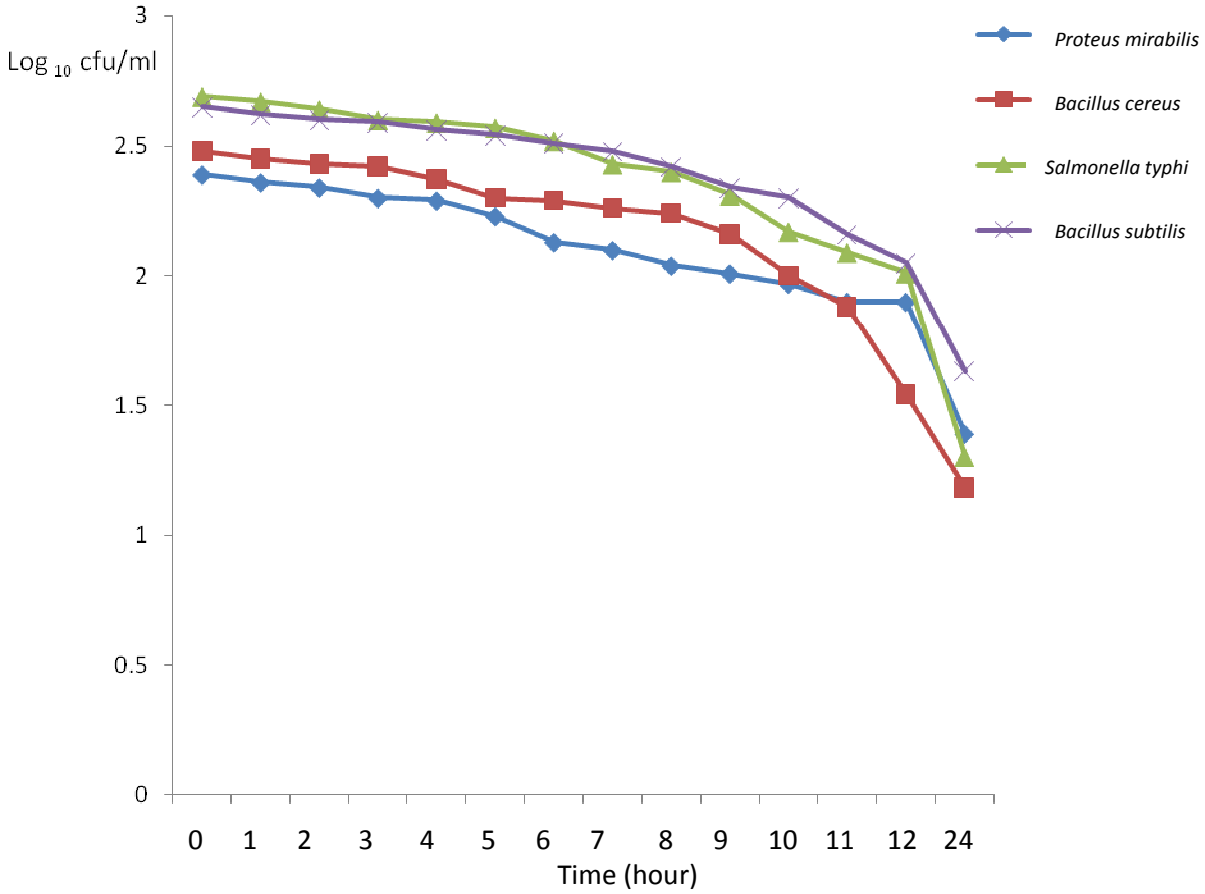

Figure 2. Rate of killng of of some bacterial isolates by *Combretum mucronatum* leaf extract.

immemorial, however, this natural endowment (plants) should be exploited scientifically to its full potential so as to give answers to our health problems.

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