ISSN 1996-3351

Asian Journal of Biological Sciences

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OPEN ACCESS Asian Journal of Biological Sciences

ISSN 1996-3351
DOI: 10.3923/ajbs.2020.98.104

Research Article
Phytochemical and Antibacterial Properties of Methanolic Extracts of *Melastomastrum capitatum* Leaves

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Abstract

Background and Objective: The emergence and spread of multi drugs resistant pathogens have enormously threatened the current antibacterial therapy. This has engendered a significant search for new sources of antibacterial substances with therapeutic properties from plants. This study was designed to evaluate the phytochemical contents of *Melastomastrum capitatum* and also determine its antibacterial potentials. **Materials and Methods:** *Melastomastrum capitatum* leaves were sun dried and grinded to powder, 150 g of pulverised leaves was placed in conical flask containing 750 mL of methanol for extraction. The bacterial isolates were tested on methanol extract of *Melastomastrum capitatum* to determine the antibacterial effects. Quantitative analysis of phytochemical constituents were carried out on the methanolic extracts of *Melastomastrum capitatum* using standard laboratory procedures. Antibiotic sensitivity test was done using disc diffusion method. **Results:** Phytochemical analysis showed that *Melastomastrum capitatum* contains tannins, flavonoids, saponins, alkaloids and cardiac glycosides. The result showed that at a high concentration of 1000 mg mL^{-1}, the plant extract inhibited the growth of *Escherichia coli* and *Streptococcus pyrogenes* while it had a bacteriostatic effect on *Staphylococcus aurues* and *Salmonella* spp. At 1000 mg mL^{-1}, methanolic extract of *Melastomastrum capitatum* leaves showed the highest zones of inhibition against *Staphylococcus aureus* (24.67±0.33 mm) and *Streptococcus pyogenes* (25.00±0.00 mm) followed by *Escherichia* and *Salmonella* which had the least. **Conclusion:** This evaluation confirmed the efficacy of the plant extract as natural antibiotic and this can be employed in the treatment of infectious diseases caused by the test clinical isolates.

Key words: Phytochemical, *Melastomastrum capitatum, Staphylococcus aureus*, methanolic extract, antibiotic resistance

Citation: D.A. Ighere, C.J. Ezenwata, D.E. Edagbo, A.A. Alowonle, E.F. Lawyer, C. Michael, N.S. Abaku, R. Oham, A.O. Dave-Omoregie and O.D. Okere, 2020. Phytochemical and antibacterial properties of methanolic extracts of *Melastomastrum capitatum* leaves. Asian J. Biol. Sci., 13: 98-104.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plant kingdom is like a treasure house which has potential drugs that are easily available, affordable, effective and have high safety profile with reduced or no side effects[1]. The World Health Organization (WHO) affirmed that medicinal plants are the best sources to obtain a variety of drugs. Plants are being used in the management and treatment of diseases and this has been in practice since the beginning of life[2]. They have been extensively studied by advanced scientific techniques and found to indeed have medicinal properties such as anticancer, antibacterial, antioxidant, antifungal, anti-rheumatic, anti-diabetic activities, analgesic, anti-inflammatory and hemolytic properties. However, some plants have not been extensively studied in pharmacognosy and thus their medicinal values are not recognized. An example of such plants is *Melastomestrum capitatum*.

Melastomastrum capitatum is a species of flowering plant in the family Melastomataceae. *Melastomastrum capitatum* is commonly found in the tropic and in Nigeria. It grows throughout the year in the edges of water channels and valleys in Ughelli North LGA and Mambilla Plateau, Sardauna Local Government area of Taraba. In these areas, it is one of the most dominant shrubs and occasionally possesses coloured leaves[3]. It is a shrubby plant which grows up to 1.25 m high usually found in swampy areas in Nigeria especially in the Mambilla Plateau, Taraba state[3]. It is locally called Belkon by the Fulani tribe in Mambilla Plateau, Otutarogo in Urhobo Delta state, Eyopinuen in Akwa Ibom and Andekerea in Bayelsa state of Nigeria. The Fulani tribe in Mambilla Plateau in Nigeria uses the leaves of *Melestomastrum capitatum* as analgesic to treat stomach pain as well as general body pain. They also use the leaves to purify the blood, alleviate diuresis and as sedatives. The leaves of *Melastomastrum capitatum* have also been said to be used as anticancer or antitumor agents. The leaves saps are used to correct pulmonary and intestinal disorders[4]. These uses have not been scientifically approved[5]. In South-South Nigeria, the leaves are used to heal wounds[3].

However, bacterial resistance is currently one of the major threats facing mankind. There is increase prevalence of antibiotic resistant bacteria emerging from the extensive use of antibiotics. This renders current antimicrobial agents insufficient to control at least some bacterial infections[6]. The emergence and rapid spread of multi-drug resistant bacteria is affecting the health industries negatively. Some of the antibiotics have been wrongly used and are no longer effective against bacterial infections and most of these synthetic drugs elicit dangerous side effects.

The search for new antimicrobial agents is an important line of research because of the resistance acquired by several pathogenic microorganisms[6,7]. Thus, to combat antibacterial resistance, there is a need to develop new, effective and safe alternative drug. This can be done by exploiting the potentials in medicinal plants.

This study was designed to evaluate the phytochemical contents of *Melastomastrum capitatum* and also determine its antibacterial potentials.

MATERIALS AND METHODS

Collection and identification of plant: The fresh leaves of *Melastomastrum capitatum* plant were collected from a swamp around ponds at Oteri (Location for collection: 5.38267'N and 6.02474'E) in Ughelli North, Local Government Area in Delta state, Nigeria. The sample was identified and authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria. This research work was conducted from May-December, 2019. The antibacterial activity test of methanol extract of *M. capitatum* leaves was carried out against four clinically important strains of bacteria which include *Staphylococcus aureus, Streptococcus pyrogenes, Escherichia coli* and *Salmonella*. Both the zones of inhibition and minimal inhibitory concentration of the extract against these microbes were determined using disc diffusion assay.

Extract preparation: The leaves were washed with distilled water to remove dirt and other contaminants before they were sun dried for two weeks. The dried leaves were crushed into powder (Classical method of Maceration) using electrical blending machine produced by Philips, model No: HR207x, Eindhoven, Netherlands. Extraction was done using 150 g of dried powdered leaves which was placed in a flask and 750 mL of methanol was added. It was allowed to stand for 48 h at room temperature[8]. The extract obtained was filtered using a muslin cloth and concentrated using a water bath (Genlab Thermal Engineers, WBH22/FL, Widnes Cheshire, UK) to remove the solvent.

Quantitative test for phytochemicals

Determination of alkaloids: Sample extract weighing 2 g was dispensed into 100 mL of 10% acetic acid. The mixture was shaken and allowed to stand for 4 h before filtration. The mixture was filtered to remove all debris and evaporated to quarter of the original volume. About 1% concentration of ammonium hydroxide (NH_4OH) was added drop wise to

precipitate the alkaloids. It was filtered with well weighed precipitate in the filter paper, oven dried at 60°C for 30 min and then reweighed[8].

Determination of saponins: Extract sample weighing 10 g was measured into a conical flash and 100 mL of 20% ethanol added to it[8]. The suspension was heated over hot bath at 55°C for 12 h with continuous stirring using a magnetic stirrer. The mixture was filtered and the residue re-extracted with another 200 mL of 2% aqueous ethanol. The combined extract was reduced to 40 mL of the original size over a water bath at about 55°C. The purification process was repeated two more times. The pH meter (Hanna instruments, HI2550 PH/ORP and EC/TDS/NaCl meter, Romania) was adjusted by adding 4 g of sodium chloride (NaCl). The solution was shaken with 60 and 30 mL portions of butanol extract and later washed twice with 10 mL of aqueous NaCl. The remaining solution was evaporated to dryness in water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was calculated in g/100 g.

Total flavonoids content determination: The total flavonoids content of the sample extract was determined by using the colorimetric assay[9]. About 0.2 mL of the extract was added to 0.3 mL 5% $NaNO_3$ at zero time. After 5 min, 0.6 mL 10% $A1C_{13}$ was added and after 6 min, 2 mL 1 M NaOH was added to the mixture followed by the addition of 2.1 mL distilled water. Absorbance was read at 510 nm against the reagent blank and flavonoids content was expressed as mg g^{-1}.

Determination of cardiac glycoside: One gram of sample was weighed into a 250 mL round bottomed flask[8]. About 200 mL distilled water was added and allowed to stand for 2 h (for autolysis to occur). Full distillation was then carried out and 150-170 mL of distillate was collected in a 250 mL conical flask containing 2 mL of 2.5% NaOH. Anti-foaming agent (tannic acid) was added before distillation. About 100 mL of the distillate containing cardiac glycoside, 8 mL of NH_4OH and 2 mL of 5% potassium iodide (KI) was added, mixed and titrated with 0.02 M silver nitrate ($AgNO_3$) using a micro burette against a black background. Permanent turbidity indicated end point.

Determination of tannin: From the sample extract, 0.5 g of the sample was weighed into 100 mL plastic bottle[8]. About 500 mL of distilled water was added and shaken for 1 h in a mechanical shaker (Blue pard, THZ-98AB, Shanghai, China). Then 5 mL of the filtrate was pipette out into a tube and mixed with 3 mL of 0.1 M Iron (III) chloride ($FeCl_3$) in 0.1 N hydrochloric acid (HCl) and 0.008 M potassium ferrocyanide $K_4[Fe(CN)_6]$. The absorbance was measured in spectrophotometer (Thermo Scientific, NanoDrop 2000, Waltham, USA) at 720 nm wavelength within 10 min.

Preparation of culture media: The media used were prepared according to the manufacturer's instructions. Muller Hinton and nutrient agars were the media used in this study[10].

Preparation of Muller Hinton Agar (MHA): Muller Hinton agar medium was prepared by dissolving 38 g of Muller Hinton agar medium in 1000 mL of distilled water according to manufacturer's instructions. It was heated with frequent agitation and boiled to dissolve the medium completely, sterilized by autoclaving at 121°C for 15 min, then the agar medium was cooled to 45°C. The agar was poured into sterile Petri-dish and was transferred inside the laminar air flow chamber to prevent contamination of the medium.

Antibiotic susceptibility test: Test organisms were subjected to antibiotics sensitivity test using the Kirby Bauer disc diffusion on prepared media. One commercial antibiotic disc was used[8]. The antibiotic disc was carefully and firmly placed on the inoculated plates using a sterile pair of forceps. The plates were inverted and incubated for 37°C for 24 h. The diameter of the zone of inhibition was measured in millimeters using a meter rule. The experiment was carried out in triplicates to minimize probability of error.

Antibacterial activity: Test isolates obtained from the University of Benin Teaching Hospital, Nigeria (Microbiology Laboratory) were used. The antibacterial activity of the plant extract against the pathogens was checked by agar well diffusion method. Cultures of pathogens were aseptically swabbed on Muller Hinton agar plates (standardized inoculums of the test bacteria adjusted to 0.5 McFarland turbidity standards). Wells of 5 mm diameter was made aseptically by cork borer in the inoculated plates and different concentrates was added into the labeled wells. The plates were incubated at 37°C for 24 h in upright position. The zone of inhibition in millimeter was recorded with the help of meter rule. The experiment was performed in triplicates to minimize probability of error[11].

Minimum inhibitory and bactericidal concentrations: To a test tube, 1 mL of each sample at different concentrations was added, 1mL of nutrient broth was added and then a loopful of

the test organism previously diluted to 0.5 McFarland turbidity standard was introduced to the tubes and observed for turbidity after incubation[11].

Bactericidal activity: To a test tube, 1 mL of 1000 mg mL^{-1} of sample was added, 1 mL of nutrient broth was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard was introduced to the tubes[11]. A loopful of broth from each of the tubes was sub-cultured into extract fresh free nutrient agar plates.

Statistical analysis: The data generated were subjected to one-way analysis of variance (ANOVA) using Genstat 12th edition analytical package as well as Non-Parametric t-test. Differences in mean were compared by Duncan's multiple range tests.

RESULTS

The quantitative phytochemical screening of *Melastomastrum capitatum* extract is shown in Table 1 with cardiac glycoside having the highest value followed by alkaloid, flavonoid, tannins and saponin.

Comparison between the antibacterial activities of *Melastomastrum capitatum* leaves extract and control (chloramphenicol) is shown in Table 2. Control sample (chloramphenicol) had higher antibacterial activities against the tested clinical isolates with zones of inhibition which ranged from 17.33±0.67 to 28.67±0.88 mm for *Staphylococcus aureus*, 18.67±0.88 to 31.00±1.53 mm for *Streptococcus pyogenes*, 19.67±0.33 to 35.67±1.86 mm for *Escherichia coli* and 14.67±0.67 to 40.33±0.88 mm for *Salmonella* sp. *Melastomastrum capitatum* extract with zones of inhibition ranged from 6.67±0.33 to 24.67±0.33 mm, 7.33±0.33 to 25.00±0.00 mm, 5.67±0.67 to 24.33±0.33 mm and 5.33±0.33 to 10.67±0.88 mm against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Salmonella* spp. at 125-1000 mg mL^{-1}, respectively. *Melastomastrum capitatum* extract had the highest zone of inhibition at 1000 mg mL^{-1} against *Streptococcus pyogenes* 25.00±0.00 mm while the control sample had highest zone of inhibition at 500 mg mL^{-1} against *Salmonella* spp. (40.33±0.88 mm).

Table 3 showed the Minimum Inhibitory Concentration (MIC) of plant extract against test organisms. *Melaslomastrum capitatum* extract had MIC against *Escherichia coli*

Table 1: Quantitative phytochemical screening of *Melastomastrum capitatum* leaves extracts

Phytochemical	Constituent (g/100 g)
Alkaloid	14.17±0.18
Tannin	10.26±0.20
Saponin	09.27±0.19
Cardiac glycoside	15.67±0.69
Flavonoid	12.39±0.58

Table 2: Comparison of the antibacterial activity of *Melastomastrum capitatum* leaves extract and control (chloramphenicol)

	Plant extract		Control (chloramphenicol)	
Organisms	Concentration (mg mL^{-1})	Zones of inhibition (mm)	Concentration (mg mL^{-1})	Zones of inhibition (mm)
Staphylococcus aureus	1000	24.67±0.33	N/A	N/A
	500	13.00±0.58	500	28.67±0.88
	250	08.00±0.58	250	25.67± 2.03
	125	06.67±0.33	125	20.00±0.58
	100	N/A	100	17.33±0.67
Streptococcus pyogenes	1000	25.00±0.00	N/A	N/A
	500	16.00±0.50	500	31.00±1.50
	500	16.00±0.58	500	31.00±1.53
	250	13.33±0.88	250	27.67±1.76
	125	7.33±0.33	125	24.67±2.17
Escherichia coli	100	N/A	100	18.67±0.88
	1000	24.33±0.33	N/A	N/A
	500	14.67±1.45	500	35.67±1.86
	250	11.33±0.88	250	35.00±0.58
	125	5.67±0.67	125	24.67±0.00
Salmonella spp.	100	N/A	100	19.67±0.33
	1000	10.67±0.88	N/A	N/A
	500	11.00±0.58	500	40.33±0.88
	250	10.33±0.88	250	36.33±0.33
	125	5.33±0.33	125	20.33±0.88
	100	N/A	100	14.67±0.67

N/A: Not available

Table 3: Minimum Inhibitory Concentration (MIC) of *Melastomastrum capitatum* leaves extract

Isolates	Concentration (mg mL^{-1})	Growth	Inference
Escherichia coli	125	-	
	110	-	MIC
	100	+	
Streptococcus pyogenes	125	-	MIC
	110	+	
	100	+	
Staphylococcus aureus	125	-	MIC
	110	+	
	100	+	
Salmonella spp.	125	-	
	110	-	
	100	-	MIC

+: Positive, -: Negative

Table 4: Bactericidal activity of *Melastomastrum capitatum* leaves extract at 1000 mg mL^{-1}

Bacterial isolate	Bactericidal activity
Escherichia coli	+
Streptococcus pyogenes	+
Staphylococcus aureus	-
Salmonella spp	–

+: Positive, -: Negative

at 110 mg mL^{-1}, *Salmonella* spp. at 100 mg mL^{-1}, *Streptococcus pyogenes* and *Staphylococcus aureus* at 125 mg mL^{-1}.

The bactericidal activities of test isolates against plant extract at 1000 mg mL^{-1} are shown in Table 4. *Melastomastrum capitatum* extract has bactericidal activity against *Escherichia coli* and *Streptococcus pyogenes*. Growth on nutrient agar plate after 24 h incubation at 36°C indicated that the plant does not exhibit bactericidal activity even at 1000 mg mL^{-1} against *Staphylococcus aureus* and *Salmonella* spp.

DISCUSSION

Melastomastrum capitatum phytochemical analysis showed the presence of tannins, flavonoids, saponins, alkaloids and cardiac glycosides. The result showed that cardiac glycosides is highest phytochemical constituent in methanol (15.67±0.69 g/100 g) extract of *Melastomastrum capitatum*. This suggested that *Melastomastrum capitatum* leaves possesses qualities that makes it suitable for enhancing proper heart functioning. Cardiac glycosides are a class of organic compounds that increase the output force of the heart and increase its rate of contractions by acting on the cellular sodium-potassium ATPase pump[12]. Their beneficial medical uses are as treatments for congestive heart failure and cardiac arrhythmias, however, their relative toxicity prevents them from being widely used[13]. This was followed by alkaloids (14.17±0.18 g/100 g), the presences of these secondary metabolites are the basis for medicinal properties of *M. capitatum* and other medicinal plants[14,15]. The least was saponins with 9.27±0.19 g/100 g. Saponins are important dietary reserves, they are glycosides in nature and have an expectorant action which is useful in the management of upper respiratory tract infections[16]. Saponins may enhance nutrient absorption and aid in animal digestion[16]. However, saponins are often bitter to taste and so can reduce plant palatability (e.g. in livestock feeds) or even imbue them with life-threatening animal toxicity[17]. Saponins exhibit antifungal properties reduce blood sugar and cholesterol by preventing the reabsorption of the cholesterol. They also have antitumor and anti-mutagenic activities and can lower the risk of human cancers by preventing cancer cells from growing. Saponins may also boost immune system and help to protect our body against viruses and bacteria.

Tannins have been shown to possess anti-carcinogenic and anti-mutagenic potentials, this is due to their anti-oxidative property which is very important in protecting the cells against cellular oxidative damage as well as lipid peroxidation[18]. Tannins also show an effective and high antioxidant property as such the presence of tannin in *Melastomastrum capitatum* is an indication that it possesses anti-carcinogenic and anti-mutagenic potentials. Tannin has been shown to inhibit the growth of many fungi yeasts, bacteria and viruses. It prevents the development of bacteria by precipitating microbial proteins and making nutritional proteins unavailable for the bacteria, thus inhibiting their growth[19].

Alkaloids present important properties with biochemical, pharmacological and medicinal effects in living organisms. Alkaloids have devised pharmacological activities and their consumption and administration at right doses are beneficial in terms of health. They have a wide range of medicinal properties including relief of pain (e.g., morphine) and analgesic (codeine) etc. The presence of alkaloids is an indication that the leaves of *M. capitatum* exhibit analgesic,

anti-inflammatory properties and has an increased potential to be effective against diseases and stress[20]. This might explain the reason for its usage as analgesic by the Fulani tribe.

Flavonoids are a ubiquitous group of polyphenolic substances present in most plants. They are known to exhibit diverse medicinal activities and are hence valuable in the prevention and treatment of many diseases[21]. The flavonoids present in *M. capitatum* promotes several health benefits such as antioxidant, antiviral, anti-cancer, anti-inflammatory and anti-allergic properties e.g., Quercetin. The cardiac glycosides may also be responsible for the antitumor and anti-cancer claim and treatment of congestive heart failure.

Staphylococcus aureus and *Streptococcus pyrogenes* exhibited the maximum zones of inhibitions at the concentration of 1000 mg mL^{-1} and were most susceptible to the methanol extract of *M. capitatum* leaves treatment. This is also the case for different concentrations of the extract. *Salmonella* has the lowest zone of inhibition even at a high concentration of 1000 mg mL^{-1}. At 250 and 500 mg mL^{-1}, the activity of *M. capitatum* against *Salmonella* increased a bit but reduced at 1000 mg mL^{-1}. This showed that *Salmonella* was less susceptible to the methanol extract of *M. capitatum* leaves. The minimum inhibitory concentration values displayed the same trends for both *Streptococcus pyrogenes* and *Staphylococcus aureus* while almost the same trend for *E. coli* and *Salmonella*.

Salmonella had the lowest MIC value which indicated that less amount of methanol extract of *Melastomastrum capitatum* leaves was required for the inhibition of the growth of *Salmonella* followed by *E. coli*.

At 1000 mg mL^{-1} of methanol extract of *Melastomastrum capitatum* leaves showed that the extract had an inhibitory effect on *E. coli* and *Streptococcus aureus* while its effect on *Staphylococcus aureus* and *Salmonella* was bactericidal.

Chloramphenicol was used as a control against the test isolates. At 500 mg mL^{-1} of chloramphenicol, *Salmonella* spp. had the highest zone of inhibition (40.33±0.88 mm) followed by *Escherichia coli* (35.67±1.86 mm). Chloramphenicol had higher zones of inhibition against the test isolates than *Melastomastrum capitatum* irrespective of the fact that the test organisms were also inhibited by the plant extract. This showed that the test isolates were more sensitive to chloramphenicol than methanol extract of *Melastomastrum capitatum*. This may be attributed to the fact that the active ingredients present in *Melastomastrum capitatum* are in crude, impure, unrefined and combined form. Whereas chloramphenicol which is a conventional antibiotic is in refined and purified form. However, if the active metabolites present in *Melastomastrum capitatum* can be extracted, purified and concentrated, it might be more effective than chloramphenicol.

CONCLUSION

The studies revealed that *Melastomastrum capitatum* contains alkaloids, tannins, flavonoids, saponins and cardiac glycosides which are responsible for its medicinal activities. The results indicated that cardiac glycosides and flavonoids are more present in *Melastomastrum capitatum* rather than other phytochemicals. Furthermore, the antibacterial study indicated that *Melastomastrum capitatum* extract was effective against the pathogens used for the study, which highlights its potential to be used as a local medicine. It is a good antibacterial agent that is capable of inhibiting the growth of various clinically important microbes. The extract of *Melastomastrum capitatum* can be used as a safe and natural alternative to antibiotics with less incidence of showing multi-drug resistance.

SIGNIFICANCE STATEMENT

This study discovered that extract of *Melastomastrum capitatum* can be used as a safe and natural alternative to antibiotics with less incidence of showing multi-drug resistance. The study revealed that *Melastomastrum capitatum* extract was effective against the pathogens used for the study, which highlights its potential to be used as a local medicine. This study will help researchers to uncover the critical area of using the extract of *Melastomastrum capitatum* as an antibiotic medicine, a plant that many researchers were not able to explore. Thus a good antibacterial agent that is capable of inhibiting the growth of various clinically important microbes has been developed.

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