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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *MICROCOS PANICULATA* LEAVES

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Abstract

The objective of this study was to determine the antimicrobial activity of methanol, chloroform and aqueous extracts of Microcos Paniculata leaves against some gram positive and gram negative bacteria such as B. subtilis, B. cereus, S. typhi, V. cholerae , P. mirabilis, E.coli, S. aureus, Serratia spp., Erwinia spp., Pseudomonas spp., Salmonella spp., Shigella boydii, B. megaterium. The in vitro antimicrobial activity was performed by agar disc diffusion method. However, maximum antimicrobial activity of the plant extracts was found against gram negative bacteria. Among the plant extracts, methanolic extract of Microcos Paniculata leaves showed highest zone of inhibition (27mm) against Salmonella spp. So, the Microcos Paniculata leaves may be a source of antimicrobial agent.

Keywords: *Antimicrobial activity, Microcos paniculata, Leaves.*

Introduction

Microcos Paniculata belongs to the family tiliaceae is a herbaceous plant that looks like a shrub or small tree and widely found throughout Bangladesh. It is locally known as 'Kathgua' or 'Fattashi' in Bangladesh. Locally various parts of the plant is used for the treatment of fever, diarrhea, dyspepsia, heatstroke, colds, hepatitis, wound healing and to kill insects. Literature review showed that Microcos paniculata has been found to have wide range of activities including neuropharmacological, larvicidal, insecticidal, free radical scavenging, antimicrobial, brine shrimp lethality, antidiarrheal, analgesic, anti-inflammatory, antipyretic, α-glucosidase inhibition, cytotoxic and nicotinic receptor antagonist activities as well as preventative effects in coronary heart disease and angina pectoris [1].

Therefore, the present study was designed to identify phytoconstituents contained in and to investigate the antimicrobial activity of aqueous extract (LWE), methanol extract (LME), and chloroform extract (LCE) of Microcos paniculata leaves.

Materials and methods

Collection and identification of plant material

M. paniculata leaves were collected from the Jahangirnagar University campus (23.8791° N, 90.2690° E), Savar, Dhaka, Bangladesh in November, 2013. Species identification was verified by Sarder Nasir Uddin, Principal Scientific Officer at the Bangladesh National Herbarium (accession number 35348). A dried specimen was deposited in the herbarium for future reference.

Preparation and extraction of plant material

Aqueous, methanolic and chloroform extraction were carried out individually by using 200 g of powdered leaves of *M. paniculata*. Fresh leaves were rinsed 3–4 times successively with running water and once with sterile distilled water. Washed plant material was then dried in the shade for a period of 7 d. The dried plant material was then ground by using a laboratory grinding mill (MACSALAB 200 Cross Beater, Eriez, Erie, Pennsylvania, U.S.A.) and passed through a 40-mesh sieve to get fine powder. Powdered leaves (200 g) were extracted in 2 L of water, methanol and chloroform by using a soxhlet apparatus and a hot extraction procedure. Whatman No.1 filter papers were used to filter the liquid extract. The filtrate was then dried in a hot air oven (BST/HAO-1127, Bionics Scientific Technologies Pvt. Ltd., Delhi, India) at 40 ℃. The extraction yield of LWE, LME and LCE were 5.08% (w/w), 14.11% (w/w) and 9.67% (w/w) respectively, which were stored at 4 ℃ for additional studies.

Preliminary phytochemical screening

Freshly prepared *M. paniculata* plant extracts (LME and LCE) were subjected to different qualitative tests like Molisch's test for carbohydrate; Fehling's test for reducing sugars; alkaloid test by using Mayer's, Dragendorff's and Wagner's reagents; frothing test for saponin; $FeCl_3$ test for tannin; alkali test for flavonoids; Salkowski's test for triterpenoids; Baljet test for finding glycosides. These tests were confirmed through characteristic color changes [2].

Test microorganisms and preparation of stock culture

Department of Microbiology, Jahangirnagar University, Savar, Dhaka, Bangladesh supplied four gram positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*) and nine gram negative bacteria (*Shigella boydii*, *Escherichia coli*, *Salmonella typhi*, *Erwinia spp.*, *Vibrio cholerae*, *Proteus mirabilis*, *Serratia spp.*, *Salmonella spp.*, *Pseudomonas spp.*) that were verified by gram staining and sub culturing in appropriate selective media on which the action of the plant extracts were tested.

Preparation of standard culture inoculum of test microorganisms

2 ml nutrient broth was used for inoculating three or four isolated colonies and the inoculated colonies were incubated by WHO's recommendation as long as the growth in the broth was equivalent with 0.5% Mac-Farland standard.

Antimicrobial assay

For the initial screening of test bacteria, the agar disc diffusion is utilized as an *in vitro* method [3]. Necessary Petri plates were prepared, following autoclave technique for 15 minutes at 121°C and the Laminar air flow were used to cool them. 20 ml of media was transferred into each sterile Petri plates aseptically and solidification was happened. By using sterile glass rod, 1 ml inoculum

suspension was spread equivalently over the agar medium to get uniform bacterial distribution. Sterile discs that were prepared instantly were loaded by required doses of plant extracts and were put gently over the media. After that, for proper diffusion, 1 hour incubation period at 5°C was completed. Again, the incubation was run for 24 hours at 37°C. Through the measurement of apparent zone of inhibition around the disc, the antimicrobial activity was documented.

Results

Phytochemical screening

Phytochemical screening of the LME and LCE showed the presence of primary as well as many secondary metabolites, or phytoconstituents, which are summarized in Table 1. Moreover, glycosides were absent in both LME and LCE. In addition to, alkaloids were absent in LCE. But other phytoconstituents as like as carbohydrates, saponins, tannins, flavonoids and triterpenoids were present in both LME and LCE (Table 1).

Antimicrobial activity

The antimicrobial activity of aqueous, methanol and chloroform extracts of *Microcos paniculata* plant were investigated using disc diffusion method against selected human pathogen (*Bacillus subtilis, Bacillus cereus, Bacillus megaterium, staphylococcus aureus, Salmonella typhi, Vibrio cholerae, Proteus mirabilis, Escherichia coli, Serratia spp., Erwinia spp., Pseudomonas spp., salmonella spp., Shigella boydii*). The aqueous, methanol and chloroform extracts of the plant have shown varied degrees of antimicrobial activity against the pathogens. Dose dependent inhibition was observed by the all extracts against both gram positive & gram negative bacteria. (Table 2-4). However, LWE showed maximum zone of inhibition (18mm) against *P. mirabilis* followed by *E. coli* (17mm), *Pseudomonas spp.* (16mm), *B. cereus* (15mm), *B. megaterium* (14.5mm), *S. aureus* (13mm), *Salmonella spp.* (13mm), *S. typhi* (12mm), *V. cholera* (11mm), *Serratia spp.* (11mm), *Erwinia spp.* (11mm), *B. subtilis* (10mm) and *Shigella boydii* (10mm) (Table 2). On the other hand, LME showed maximum zone of inhibition (27mm) against *Salmonella spp.* followed by *Pseudomonas spp.* (23mm), *V. cholera* (21mm), *Serratia spp.* (21mm), *S. aureus* (20mm), *Erwinia spp.* (20mm), *S. typhi* (19mm), *P. mirabilis* (19mm), *B. subtilis* (18mm), *Shigella boydii* (18mm), *E. coli* (17.5mm), *B. cereus* (17mm) and *B. megaterium* (12mm) (Table 3). But, LCE showed maximum zone of inhibition (25mm) against *Salmonella spp.* and *S. aureus* followed by *V. cholera.* (23mm), *P. mirabilis* (22mm), *E.coli* (21mm), *B. subtilis* (19mm), *Pseudomonas spp.* (18mm), *Shigella boydii* (18mm), *S. typhi* (17mm), *B. megaterium* (17mm), *B. cereus* (16mm), *Serratia spp.* (15mm) and *Erwinia spp.* (15mm) (Table 4).

Discussion

Phytochemical components are identified as bioactive compounds of plant extracts and may be responsible for the diverse activities when herbs are used medicinally. Secondary herbal metabolites have influence on the medicinal and pharmacological actions of medicinal herbs. Primary metabolites (e.g., amino acids, monosaccharides, nucleic acids, polysaccharides, proteins, lipids) are present in almost all plant species, whereas secondary metabolites are found in fewer plant species; in the plant, these compounds provide defences against herbivores and pathogens, attract pollinators and fruit dispersers, give mechanical support, absorb harmful ultraviolet radiation and reduce the growth of nearby competing plants. Alkaloids, phenolics (simple phenolics and flavonoids), terpenoids, fatty acids, glycosides, waxes and their derivatives are examples of secondary metabolites that can have medicinal properties. In the field of drug discovery and development, the preliminary screening of secondary metabolites facilitates the recognition of bioactive compounds [2].

The variation in the antimicrobial property of the *M. paniculata* extracts might be due to the presence of various secondary metabolites that were found during phytochemical analysis. There is a possibility that due to insufficient antibacterial constituents, some extracts were less effective against certain bacteria. From the existing study, it is clear that the gram negative bacteria were inhibited more than the gram positive bacteria by the all extracts of *M. paniculata*. Plant's secondary metabolites include phytochemical components such as phenols, alkaloids, saponins, flavonoids, tannins and a number of aromatic compounds which provide a defense mechanism against insects, herbivores and many microorganisms through

anticipation. Different mechanisms are engaged for exerting the antibacterial action of bioactive compounds. When microbes infect plants, then flavonoids are synthesized by plants which are hydroxylated phenolic substances. And these flavonoids act as effective antibacterial agents covering a variety of bacteria that was established by *in vitro* method. Possibly they perform their actions through complex formation with soluble and extracellular proteins along with bacterial cell walls also. Saponins show their antibacterial actions by causing leakage of certain enzymes and proteins from the cell. Triterpenoids exhibit antibacterial activity against gram positive bacteria, block cell division by inhibiting DNA synthesis and macromolecular synthesis in *Bacillus subtilis*. In case of gram negative bacteria, the lipopolysaccharide layer creates a barrier for the entry of most of the compounds. Gram negative bacteria's external membrane creates a permeable barrier for stopping access of bulky polar substances into the cell. However, protein channels which are aqueous in nature regarded as porins, can facilitate the entry of small polar substances as well as many small hydrophilic antibiotics into the periplasmic space of Gram negative bacteria. Different gram negative bacteria vary according to the number and size of porins. *Pseudomonas aeruginosa* lacks the classical high permeability porin channels and shows resistant to a wide range of antibiotics. In case of gram negative bacteria, some antibiotics can pass through the porins by passive diffusion and other can pass across the cytoplasmic membrane via an energy dependent active transport system. For example, the aminoglycosides are transferred across the cytoplasmic membrane, which depend on electron transport because of necessity for a membrane electrical potential. Metabolic energy is needed for some antibiotics to enter into the gram positive bacteria, but the mechanism is not clear [4]. The observed plant extracts showed antimicrobial activity against most of the gram negative bacteria which may be due to the presence of porin channels and the usage of either active transport or passive diffusion.

Conclusion

In conclusion, the extracts of *Microcos paniculata* act as antimicrobial agent. However, detailed studies are required for determining and isolating the antimicrobial agents present in the plant extracts of *Microcos paniculata* leaves.

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Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Table 1: Phytochemical screening of LME and LCE.

Phytoconstituents	Test name	Observation of various extracts	
		LME	LCE
Carbohydrates	Molisch's test	+	+
	Fehling's test	+	+
Alkaloids	Mayer's test	+	-
	Wagner's test	+	-
	Dragendorff's test	+	-
Saponins	Frothing test	+	+
Tannins	$FeCl_3$ test	+	+
Flavonoids	Alkali test	+	+
Triterpenoids	Salkowski's test	+	+
Glycosides	Baljet test	-	-

+: presence of specific phytoconstituents; -: absence of specific phytoconstituents

Table 2: Antimicrobial activity of LWE.

Diameter of zone of inhibition (mm)			
Organisms	**2 mg/disc**	**4 mg/disc**	**6 mg/disc**
B. subtilis	-	7	10
B. cereus	-	7	15
S. typhi	-	7	12
V. cholera	-	7	11
P. mirabilis	-	10	18
E. coli	6.5	11	17
S. aureus	-	7	13
Serratia spp.	-	8	11
Erwinia spp.	-	9	11
Pseudomonas spp.	-	7	16
Salmonella spp.	-	7	13
Shigella boydii	-	8	10
B. megaterium	-	9	14.5

Table 3: Antimicrobial activity of LME.

Diameter of zone of inhibition (mm)			
Organisms	**2 mg/disc**	**4 mg/disc**	**6 mg/disc**
B. subtilis	6	12	18
B. cereus	-	12	17
S. typhi	6.5	14	19
V. cholera	7.5	15	21
P. mirabilis	-	13	19
E. coli	-	13	17.5
S. aureus	6.5	16	20
Serratia spp.	-	14	21
Erwinia spp.	-	13	20
Pseudomonas spp.	7.5	15	23
Salmonella spp.	11	20	27
Shigella boydii	6	12	18
B. megaterium	-	10	12

Table 4: Antimicrobial activity of LCE.

Diameter of zone of inhibition (mm)			
Organisms	**2 mg/disc**	**4 mg/disc**	**6 mg/disc**
B. subtilis	7.5	13	19
B. cereus	-	11	16
S.typhi	-	14	17
V. cholerae	8.5	16	23
P. mirabilis	7.5	15	22
E.coli	7.5	15	21
S. aureus	8.5	18	25
Serratia spp.	-	11	15
Erwinia spp.	-	10	15
Pseudomonas spp.	7	14	18
Salmonella spp.	8.5	19	25
Shigella boydii	6.5	13	18
B. megaterium	-	13	17