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Research Article

Preliminary Phytochemical and Antimicrobial Studies of *Nulembo Nucifera* Rhizome

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ABSTRACT
The study focused on phytochemical and antimicrobial activity of *Nulembo nucifera* rhizome. The rhizome extracts of the plant was taken for the phytochemical screening to identify the phytochemicals present in it. The extracts of the rhizome were evaluated for antimicrobial activity against *different* Gram + ve and gram – ve bacteria by disc diffusion method. The DCM extracts showed significant antimicrobial activity against all the strains as compared to the standard drug. The extracts showed high amount of antimicrobial activity.

Keyword: Antimicrobial activity, phytochemical screening, *Nulembo nucifera*

INTRODUCTION

According to World Health Organization (WHO), medicinal plants are the best source to achieve a wide variety of drugs. Now a days it has been observed that in the developed country near about 80% of plants are used traditionally as a medicine. Those plants have been further investigated for better understanding of their medicinal properties. In the worldwide many plants have been investigated for their antimicrobial properties by a number of researcher's . Medicinal plants play an effective role for the benefit of the human being. Natural products are played an important role in both drug discovery and chemical biology. Although some specific plant compounds contain some specific therapeutic benefits, many herbs contain lots of active constituents that, together, combine to give the plant its therapeutic effectiveness. Any part of the plant may contain any active components [1]. Over the past few years more emphasized has been given to natural products as sources of new antibacterial agents. After traditional medicine has been accepted as an alternative system of health care and the development of microbial resistance to the antibiotics has supported a lot to the researches to find out the potent antimicrobial activity of medicinal plants [2]. By using different parts of the plant extract obtained from the roots, barks, stems, leaves and seeds the practice of herbal medicines can be done in Medical uses . Medicinal plants were used as powerful antimicrobial agents because it contains a variety of chemical constituens that can be resistant to various microbes. From the plant it is possible to synthesize aromatic substances such as phenolic, (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids) and some other effective

bioactive metabolites and these bioactive metabolites showed plant defense mechanisms against microbes[3].

It is the most promising advances in the area of drug discovery, discovering new entities or novel uses of the previously existing compounds with known efficacy and without producing any side effects. These types of bioactive metabolites combined with other commonly available antibiotics can destroy the drug resistant bacteria. Based on the traditional claim *Nelumbo nucifera* plants and due the lack of scientific investigation of its potent antimicrobial properties, the main objective of this investigation was to estimate the antimicrobial properity of both the plants [4].

MATERIALS AND METHODS

Collection and extraction of plant material

The rhizomes of *Nulembo nucifera* were collected from the localities in Guwahati, Kamrup (M),Assam during the month of march 2017. The N.nucifera were collected, shade dried, powdered mechanically and sieved through no. 20 mesh sieve. About 100g of powdered rhizome is extracted successively with petroleum ether (PEL), 60° - 80° C and then with dichloromethane (DCM), n-butanol (NB), ethyl acetate (EEL) and methanol (MEL). The extract collected was filtered and evaporated using rotary evaporator and stored in vacuum desiccators. The percentage yield of the extract is listed in table 1.

Phytochemical screening of the extracts:

Phytochemical screening were carried out for NB, DCM, MEL extract of *N.nucifera rhizome* for the presence of phytochemical constituent like alkaloid, glycoside, tannin, amino acid, steroid, protein, flavonoid etc [5,6].

Antimicrobial activity

Nutrient Agar Media

Bacteriological media is a wide range of types. Nutrient Agar is a complex medium as it contains ingredients with unknown amounts or types of nutrients. Nutrient Agar contains Beef Extract (0.3%), Peptone (0.5%), Agar (1.5%) and sodium chlorite (0.5%) in water. Beef Extract is commercially prepared dehydrated form of autolyzed beef and is supplied in the form of a paste. Peptone is the casein (milk protein) that has been digested with the enzyme pepsin. Peptone is dehydrated and supplied as powder. Peptone and Beef Extract contain a mixture Amino and peptides of acids Beef Extract also contains water soluble digest products of all other macromolecules (nucleic acids, Fats, polysaccharides) as we as vitamins and trace minerals. Although we know and can define Beef Extract in these terms, each batch cannot be chemically defined. There are many media ingredients which are complex: yeast extract, tryptone, and others. The advantage of complex media is that they support the growth of wide range of microbes. Agar is purified from red algae in which it is an accessory polysaccharide (polygalacturonic acid) of their cell walls. Agar is added to microbial media only as a solidification agent. Agar for most purpose is not nutrient value. Agar is an excellent solidification agent because it dissolves at near boiling but solidifies at 45^{0}C. Thus, one can prepare molten (liquid) agar at 45^{0}C, mix cells with it, and then allow it to solidify thereby trapping living cells. Below 45^{0}C is a solid and remains so as the temperature is raised melting only when >95^{0}C is obtained.

Procedure

- Beef Extract, peptone were accurately weighted out and in 1000ml of distilled water beef extract, peptone are added.
- pH was checked by using pH meter (pH 7.4), and finally Agar was added to the flask.
- Boil the media to dissolve & allow it to cool a few minutes.
- The media is placed an autoclave for sterilization, while the agar is still warm, but not hot for 20 minutes.
- Then the media is being taken from the autoclave and pour it is into the Petridis with labeled as test and standard.
- The antimicrobial activities of the test agents were determined by measuring the diameter of the zone of inhibition.

Materials

Petri dish, Beef extract, Peptone, Agar, Ethanol, Distilled water.

Microorganisms

The species of bacterial organisms were *S. aureus, E.coli, K. pneumoniae*, *V. cholera, P. aeruginosa and B. subtilis* used. These cultures of bacteria were maintained on nutrient agar slants a in GIPS microbiology laboratory at 4^{0}C.

Standard Drug
Chloramphenicol.

Preparation of impregnated discs of extract and standard antibiotics
The discs were prepared having 7.25 diameter by punching of Whatman No.1 and these were sterilized by using dry heat at 160ºC for an hour in screw capped Bijou bottles. The DCM extracts of *N.nucifea* was weighed and dissolved in sterile distilled water to make the concentration 128-2000 µg/ml. Similarly the stock solution having the concentration ranges from 0-1000 µg/ml of the control antibiotic (Chloramphenicol) and isolated compound were prepared by dissolving in sterile distilled water. All the stock solutions were then kept at 4ºC and used for three months. The antibiotic impregnated discs were prepared by adding 1.0 ml of the stock solutions of the antibiotic separately to each bottle of 100 discs. The procedure was repeated for preparation of the plant extracts and their isolated compounds containing discs. The discs were used in wet condition and it has to be stored at 4^0C further use. The discs can retain their potency by keeping it at screw capped bottles for at least three months [7, 8].

Antimicrobial activity of DCM extract of *N.nucifea* and isolated compound
Microbial sensitivity tests were performed by disc diffusion method. Antibacterial activity was done on nutrient agar media. 1 ml of bacteria suspension was uniformly spread on the sterile nutrient agar media Petri dish. 1.5mg of each sample was dissolved in 10 ml of the respective fractionation solvents. Sterile filter paper disc (Whatman no.1, diameter 6mm) was soaked with the extract solution and the solvent allowed to dry. The disc was placed on the nutrient agar media Petri dish inoculated with bacteria suspension and kept at 4^0C for 48 hours to allow the extracts to diffuse into the media. The Petri dish was then placed in an incubator for 24 hours at 37^0C. At the end of the incubation period, the inhibition diameter was measured using calipers and expressed in millimeters. Chloramphenicol was used as standards. Positive antibacterial was established by the presence of measurable zones of inhibition. [9,10].

Minimum Inhibitory Concentration (MIC)
The MICs were determined by using standard agar dilution method . The solution was prepared by dissolving the crude extract in 0.5ml of DMSO (Dimethylsulpoxide) and then the solution was diluted by adding sterile distilled water. Then the drug solution was to the different test tube containing molten nutrient agar media to get the final concentrations of 0 – 128 µg/ml and subsequently increasing the concentration upto 2000 µg/ml. The concentrations of the tubes were mixed thoroughly by adjusting the pH to 7.2 to 7.4 and finally poured the substances into sterile Petri dishes. By using bacterial planter (10µl) bacterial cell suspensions were inoculated on the plates and they were incubated at at 37°C±2°C for 18 h. The final number of cfu inoculated onto the agar plates was 10^5 for all strains. After incubation for 18hrs the plate which did not show any visible growth The lowest concentration of the plate, was considered as MIC. The agar plate containing only sterile distilled water and Chloramphenicol was considered as positive and negative control respectively.

RESULTS AND DISCUSSION
Results of percentage of yield and phytochemical screening are reported in table 1 and 2.

Table 1: Percentage yield of various extracts

Sl no	Solvent used	Percentage of yield
1	Petroleum Ether	1.9 % w/w
2	Ethyl acetate	3.4 % w/w
3	Dichloromethane	18 % w/w
4	n-Butanol	6% w/w

PEL: pet ether extract of N.nucifera rhizome; CEL: chloroform extract of N.nucifera rhizome; EEL: ethyl acetate extract of N.nucifera rhizome; MEL: methanolic extract of N.nucifera rhizome.

Table 2: Results of preliminary phytochemical screening

Sl.No	Test	Pet. ether	DCM	n-Butanol	Eth.Acetate
1	Alkaloid	-	-	-	-
2	Flavonoids	-	-	-	-
3	Saponin	-	-	-	-
4	Steroid	-	-	+	-
5	Tanin	-	-	+	+
6.	Terpinoids	-	+	-	-
7.	Carbohydrate	-	-	-	-
8.	Glycosides	-	-	-	-

After performing the antimicrobial activity of DCM extracts of *N.nucifera* rhizomes and isolated compound it has been found that out of 30 bacteria, at a concentration of 128-512 µg/ml of the DCM extract the growth was inhibited by of 17 isolates, but at a concentration of 1000 µg/ml the growth was inhibited by 11 isolates, while the remaining growth was inhibited by 02 isolates at a concentration of >2000 µg/ml. While performing the MICs tests it has been found that out of 13 Gram-positive bacteria 10 strains were sensitive between 128 and 256 µg/ml (Zone diameter 14-18 mm); while out of 17 Gram-negative bacteria 15 strains were sensitive between 256-512 µg/ml (Zone diameter 10-16 mm). Hence, it can be concluded that the antimicrobial activity of the DCM extracts of *N.nucifera* rhizome was showed against both Gram-positive and Gram-negative bacteria. In case of isolated compound it has been found that out of 13 Gram-positive bacteria 12 strains and15 strains out of 17 strains of Gram-negative bacteria were sensitive between 64-512µg/ml (Zone diameter 14-20 mm) concentration which showed excellent symptoms of potent antimicrobial agent .From the above study it can be concluded that all the isolates were sensitive at 128-256 µg/ml concentration of the isolated compound . The results were reported on to the table 3 and 4.

Table 3: Minimum inhibitory concentration (MIC± SD of three replicates) at 600nm OD of DCM extract of *N.nucifera*

Pathogens	Number Of strain	MIC of rhizome extracts (µg/ml)					Zone of inhibition (mm)	MIC of lincomycin (µg/ml)						
		128	256	512	1000	>2000		0.25	0.5	8	64	128	256	>1000
S.aureus	5	01	01	01	02	-	++	-	01	-	01	02	01	-
K. pneumoniae	7	01	01	-	01	01	++	01	01	-	01	02	01	01
E.coli	4	01	01	01	01	-	++	-	-	-	-	01	02	01
V. cholera	6	-	02	03	03	-	++	01	01	02	01	-	01	-
B. subtilis	3	-	01	01	02	-	+	-	-	-	01	01		01
P.aeruginosa	5	01	-	01	02	01	++	01	-	01	01	02		-
Total	**30**	**04**	**06**	**07**	**11**	**02**		**03**	**03**	**03**	**05**	**08**	**05**	**03**

Table 4: Minimum inhibitory concentration (MIC± SD of three replicates) at 600nm OD of isolated compound

Pathogens	Number Of strain	MIC of Compound 2 (µg/ml)								zone of inhibition (mm)
		0.25	0.5	8	64	128	256	512	>1000	
S.aureus	5	-	-	-	01	02	01	01	-	++
K. pneumoniae	7	-	-	-	01	01	02	02	01	+
E.coli	4	-	-	-	-	01	02	01	-	++
V. cholera	6	-	-	-	-	01	02	02	01	++
B. subtilis	3	-	-	-	-	01	01	01	-	++
P.aeruginosa	5				01	02	01		01	++
Total	30				03	08	09	07	03	

CONCLUSION

From these investigations, it may be concluded that the DCM rhizome extract of N.nucifera showed potential antimicrobial activity against both Gram-positive and Gram-negative bacteria. From the above study it can be concluded that all the isolates were sensitive at 128-256 µg/ml concentration of the isolated compound.

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