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Research Article

STUDY OF PHYTOCHEMICAL AND ANTIMICROBIAL POTENTIAL OF THE LEAVES OF *NILGIRIANTHUS CILIATUS* LINN

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ABSTRACT: The present study was carried out to identify the phytochemical constituents and to evaluate antibacterial and antifungal activity of *Nilgirianthus ciliatus* Linn using ethanol, chloroform, petroleum ether and aqueous extracts on selected four bacterial pathogens such as *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and two fungal strains *Aspergillus niger* and *Candida albicans* (Fungus-unicellular/ multicellular yeast). For the antimicrobial test, cup plate method was used and the zone of inhibition was measured in mm. The extracts showed significant antimicrobial activity and were compared with Amoxicillin and Ketoconazole. The chloroform extract showed the higher antibacterial activity with a zone of inhibition of 23mm against *gram negative* pathogens and minimum 15mm against *Aspergillus niger* from 150mcg/ml. Ethanolic and petroleum ether extracts showed similar activity with an inhibition zone of 12 and 11 mm against fungal strains *Candida albicans* and *Aspergillus niger* respectively from 150mcg/ml. The phytochemical analysis revealed the presence of alkaloids, carbohydrates, flavonoids, saponins; proteins and tannins. The powdered leaves were analyzed for various physico-chemical constants also.

Key words: Phytochemical, Antimicrobial, *Nilgirianthus ciliatus,* Alkaloids, Flavonoids, Saponins, Cup plate method.

INTRODUCTION

Nilgirianthus ciliatus (Acanthaceae), synonym *Stobilanthus ciliatus* is a shrub of 1-2 meter height. It is found distributed in southern part of India and Andaman islands. (Warrier *et al*., 1994). Traditionally the roots are used in the treatment of neurological disorders such as paraplegia, sciatica, glandular swellings, ulcers and oedema. The whole plant is used in the treatment of poisonous affections, leprosy, other skin diseases, cough, tooth-ache, jaundice, dropsy and rheumatism (Warrier *et al*., 1994). Various pharmacological activities like anti-diuretic, diaphoretic, anti-inflammatory activity (Thomas*et al*., 2000) have been reported. The objective of the present study was to evaluate antimicrobial potential of different extracts of the leaves of *Nilgirianthus ciliatus* Linn.

MATERIALS AND METHODS

Preparation of leaf extracts

Fresh leaf of *Nilgirianthus ciliatus* was collected from Ramapuram, Malappuram (Dist), Kerala. They were authenticated by Dr. Philomina, Professor and Head of Botany, Nirmalagiri College, Koothuparmba, Kannur.

Preparation of powder

The leaves of plants were dried under shade. These dried materials were mechanically powdered and stored in an airtight container. These powdered leaves were used for further analysis.

Extraction of plant material

The powdered leaf of *Nilgirianthus ciliatus* was subjected to successive solvent extraction in Soxhlet extractor with ethanol, water, petroleum ether and chloroform. The respective extracts were concentrated by vacuum distillation and dried in open air.

Phytochemical studies

The preliminary phytochemical studies were performed for testing the different chemical constituents present in extracts according to Kokate (1994) and Kokate *et al*., (1995).

Test organisms

The bacterial cultures of *Escherichia coli, Pseudomonas aeruginosa (gram negative), Bacillus subtilis, Staphylococcus aureus (gram positive),* and the fungal strains were collected from Al Shifa Hospital, Perinthalmanna, Kerala, India.

Antibacterial Studies

Bacterial Media: Muller-Hinton agar medium (pH 5.6 ± 0.2)

The medium was prepared by dissolving the specified quantities of the dehydrated medium (Hi-media) in purified water. The medium was distributed in 4 ml quantities into test tubes. The test tubes were closed with cotton plugs and were sterilized by autoclaving at 121°C (15 lbs psig) for 15 minutes. The contents of the test tubes were poured into sterile petri dishes under aseptic conditions and allowed to solidify.

Antifungal Studies

Fungal media: Sabouraud's agar medium (pH 5.4 ± 0.2)

Sabouraud's agar medium was prepared by dissolving specified quantities of dehydrated medium (Hi-media) in purified water. The medium was distributed in 4 ml quantities into test tubes. The tubes were closed with cotton plug and sterilized by autoclaving at 121°C (15 lbs psig) for 15 minutes. The sterilized media were poured into sterile petri dishes and allowed to solidify.

Cup plate method

Each petri dish was inoculated with one of the bacterial cultures and fungal strains. The inoculated plates were bored with 6mm diameter sterile cork borer. The extracts were poured into each cup, one cup was filled with standard drug, and one was filled with DMF. All the plates were kept in the refrigerator for 30 minutes and incubated at 37°C for 24 hours for bacterial pathogens and 48 hours for fungal strains. Diameter of the zone of inhibition was measured in mm and the average diameter for each sample was calculated. The diameter obtained for the test samples were compared with that of the diameter produced by standard drugs. The diameter of zone of inhibition is proportional to the antimicrobial activity of the sample.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The results of phytochemical screening are given in table1. The phytochemical screening of different extracts revealed the presence of alkaloids, carbohydrates, flavonoids, saponins, proteins and tannins.

Antimicrobial activity

The crude extracts such as chloroform, petroleum ether, aqueous and ethanol at a concentration of 150 mcg were studied for antibacterial and antifungal activity by cup plate method. The extracts exhibited potential inhibitory activity against gram positive and gram negative bacteria; also exhibited remarkable activity against fungus strain. Among the different samples, chloroform extract showed higher inhibitory effect with maximum zone of inhibition against different pathogens. Aqueous, petroleum ether and ethanol extract showed moderate activity. The results are given in table 2.

Physico-chemical constants

The powdered leaves were analyzed for various physico-chemical constants like total ash value, alcohol soluble extractive value, water-soluble ash value, acid-insoluble ash value and loss on drying. The results are given in Table3.

Table-1: **Results of Qualitative Tests for Phytoconstituents in leaves extract fractions**

S.No	Tests	Extracts			
		Ethanol extract	**Pet.Ether extract**	**Chloroform extract**	**Aqueous extract**
1.	**Alkaloids**	**+ve**	**+ve**	**+ve**	**+ve**
2.	**Carbohydrates**	**+ve**	**+ve**	**+ve**	**+ve**
3.	**Flavonoids**	**+ve**	**+ve**	**+ve**	**+ve**
4.	**Glycoside**	**-ve**	**-ve**	**-ve**	**-ve**
5	**Protein**	+ve	+ve	+ve	+ve
6.	**Resins**	-ve	-ve	-ve	-ve
7.	**Saponins**	+ve	+ve	+ve	+ve
8.	**Tannins**	+ve	+ve	+ve	+ve
9.	**Starch**	-ve	-ve	-ve	-ve
10.	**Steroids**	-ve	-ve	-ve	-ve

+ve = Present, - ve = Absent.

Table 2: Data of antimicrobial activity of different extracts. (Diameter of the inhibitory zones (in mm))

Sl. no	Compound	Conc. mcg/ml	*S.aureus*	*B.subtilis*	*Paeruginosa*	*E. coli*	*C.albicans*	*A. niger*
			Diameter of the inhibitory zones (in mm)					
1	Ethanolic extract	150	12	14	13	11	12	11
2	Pet. ether extract	150	16	11	08	12	12	11
3	Aqueous extract	150	12	14	12	13	10	09
4	Chloroform extract	150	18	18	19	23	16	15
5	Amoxicillin	150	22	23	20	24	_	_
6	Ketoconazole	150	_	_	_	_	20	19

S.*aureus- Staphylococcus aureus****B.****subtilis- Bacillus subtilis, **P.****aeruginosa-Pseudomonas aeruginosa **E.** coli- Escherichia coli **C.** albicans- Candida albicans **A.** niger- Aspergillusniger*

Table 3: Pharmacognostic study of leaf of *Nilgirianthus ciliatus*

S.No	Parameters	Percentage yield (%w/w)
1.	Total ash value	26
2.	Alcohol soluble extractive value	20
3.	water-soluble ash value	83.87
4.	Acid insoluble ash value	28.84
5.	loss on drying	13.33

CONCLUSION

The result of the qualitative phytochemical screening revealed the presence of alkaloids, carbohydrates, flavonoids, saponins, proteins and tannins. The chloroform extract exhibited higher antimicrobial activity with maximum zone of inhibition against different pathogens.

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