https://doi.org/10.46344/JBINO.2021.v10i03.05

PHYTOCHEMICAL & ANTIBACTERIAL ACTIVITY OF SIDDHA DRUG ALLI CHOORNAM (*NYMPHEA NOUCHALI BURM.F*)

Dr.Janani Syamaroopa Jnanathapaswini[1], Dr.Manoharan.A[2]

[1] PG scholar, Government Siddha Medical College, Palayamkottai

[2] Vice Principal, Government Siddha Medical College, Palayamkottai

ABSTRACT

The Alli (*Nymphea nouchali Burm.f*) is an aquatic herb, under the family Nymphaeaceae. As per Siddha classical literatures this plant possess astringent, refrigerant, antimicrobial actions. The aim of this study was to evaluate the phytochemical and antibacterial activities of the crude extract of rhizome and flowers of Alli (*Nymphea nouchali Burm.f*). Screening was performed for Carbohydrate, Alkaloids, Flavonoids, Glycosides, Proteins, Terpenoid, Tannin & Saponin. Antibacterial activity was made by agar disk diffusion test. The phytochemical screening showed positive results for Carbohydrate, Alkaloids, Flavonoids and tannins in the concentration of *68±0.44mg/100g, 51±0.88 mg/100g, 38±0.49 mg/100g*, and 41±0.23 mg/100g respectively. N.nouchali extract found to be effective with concentration of (1mg/ml) against *S.aureus, E.coli, P.vulgaris, S.mutans & Klebsilla* species suppressing their growth with inhibition zones of *14mm,13mm,11mm,10mm,8mm* respectively. Alli (*Nymphea nouchali Burm.f*) demonstrated the presence of secondary metabolites which are known for their medicinal values.

Key words: Phytochemicals, Antimicrobial, Nymphaeaceae, Siddha ,Nymphea nouchali

Introduction

People all over the world are in search of harmless and safe remedies to their chronic diseases. India is very rich in herbal & medicinal plant wealth with suitable geoclimatic conditions. It has well documented & well practical knowledge about traditional herbal medicine. Discovery of drug requires a systematic and well-designed approach on the correct plant to reach the expected goals in expected time. The bacterial agents including *Klebsilla species, Staphylococcus aureus, Pseudomonas aeruginosa, Escheria coli, Proteus vulgaris, and Streptococcus mutans* cause multiple human infections. The novel evolution of antibiotic resistance & toxicity hold the use of antimicrobial drugs. The aim of the study is to evaluate the important phytochemical constituents and antimicrobial activity of Alli chooranam (*Nymphea nouchali Burm.f*).

2. Materials and Methods

2.1. Collection and Authentication of Plant

The flower & rhizome of Alli (*Nymphea nouchali Burm.f*) freshly collected from various places of Kerala. Identified and authenticated by the Medicinal Botanists at Government Siddha Medical College and Hospital, Palayamkottai. This herb purified according to the suitable procedure methods described in Siddha classical literature. The drug is dried and subjected to .size reduction to get uniform coarse powder.

2.2. Qualitative analysis phytochemical constituents in aqueous extract of the test drug

The preliminary qualitative analysis of phytochemicals to identify the secondary metabolites present in aqueous extract flower and rhizome of *Nymphea nouchali Burm.f*.

Tests for Carbohydrate, Glycosides, Steroids, Alkaloids, Flavonoids, Tannins, Saponin, Phenol, Protein, and Triterpnoids were carried out by Benedicts test, Keller – Killiani test, Salkowskitest, Mayers test, Shinoda test, Lead acetate test, Foam test, Biuret test, Ferric chloride test respectively.

2.3. Quantitative analysis phytochemical constituents in aqueous extract of the test drug

2.3.1. Quantitative estimation of Alkaloids

To 1ml of Methanolic extract add 5 ml P^h phosphate buffer and 5ml BCG solution then shake the mixture with 4ml of chloroform. The extracts were collected in a 10ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with atropine equivalents.

2.3.2. Quantitative estimation of Carbohydrate

The total sugar content was estimated by Anthrone method (Roe,1955).A Known amount of sample was taken ,ground well with 80% ethanol and was centrifuged at 4000 rpm. From the supernatant, 0.5ml was taken and 5ml of anthrone reagent was added. The tubes were kept in a boiling water bath for 15min.After that they were kept in a dark room for another 15 mins. The color intensity developed was read in a spectrophotometer at 650 nm.

2.3.3. Quantitative estimation of Flavonoids

Total flavonoid content was determined by aluminum chloride method using a standard 1ml of test sample and 4 ml of water were added to a volumetric flask (10ml volume). After 5 min 0.3 ml of 5% Sodium nitrite, 0.3ml of 10% Aluminum chloride was added. After 6 min incubation at room temperature, 2ml of 1M sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometric ally. Results were expressed as catechin equivalents.

2.3.4. Quantitative estimation of Tannins

The tannin contents were determined by method of Broadhurst et al., 1978 with slight modification, using tannic acid as a reference compound. One millimeter of the extract was mixed with 5mlofvanillin hydrochloride reagent. The mixture was allowed to stand for 20mins and measure the absorbance at 500nm. The standard graph was plotted for working standard tannic acid solution. (20-200µg/µl).

2.4. Antibacterial activity of aqueous extract of the drug

2.4.1. Bacterial strains

The test microorganisms used for antimicrobial analysis *Klebsilla species, Staphylococcus aureus, Pseudomonas aeruginosa, Escheria coli, Proteus vulgaris, and Streptococcus mutans* were purchased from Microbial type culture collection and Gene Bank, Chandigarh, The bacterial strains were maintained on nutrient agar.

2.4.2. Nutrient Broth Preparation

Pure culture from the plate were inoculated in to Nutrient Agar plate and subculture at 37^0C for 24 h. Inoculum was prepared by aseptically adding the fresh culture in to 2ml of sterile 0.145 mol/L saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial suspension of 1.5x 108 cfu/ml. Standardized inoculum used for antimicrobial test.

2.4.3. Antimicrobial test

The medium was prepared by dissolving 38g of Mullar Hinton Agar medium in 1000ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121^0 C for 15 mins (P^H 7.3).The autoclaved medium was cooled mixed well and poured petriplates (25 ml/plate) were swabbed with pathogenic bacteria culture via *Klebsilla species, Staphylococcus aureus, Pseudomonas aeruginosa, Escheria coli, Proteus vulgaris,* and *Streptococcus mutans*. Finally the sample disc was then placed on the Mullar Hinton Medium and the plates were kept for incubation at 37^0C for 24 hrs. At the end of the incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimeters. The size of the zone of inhibition was measured in millimeters. The absence of zone of inhibition was interpreted as the absence of activity (Kohner et al., 1994;Mathabe et al.,2006).

3. Results & Discussion:

Present study reveals that the aqueous extract of Alli (*Nymphea nouchali Burm.f*) showed the presence of tannins, Flavonoids, carbohydrate and Alkaloids but was negative for protein, glycoside, terpenoid, steroid, phenol & saponin.

Quantitative phytochemical estimation of secondary metabolites is summarized Table.1 in which the average carbohydrate, Alkaloid, Flavonoids, Tannin contents were estimated as 68±0.44 mg/100g, 51±0.88 mg/100g, 38±0.49 mg/100g, & 41±0.23 mg/100g respectively.

Table.1.Quantitative phytochemical estimation of secondary metabolites

Test Name	Result
Carbohydrate(mg/100gram)	68±0.44
Alkaloid(mg/100gram)	51±0.88
Flavanoid(mg/100gram)	38±0.49
Tannin(mg/100gram)	41±0.23

The measurement of antibacterial activity of flower and rhizome of extract of Alli (*Nymphea nouchali Burm.f*) are presented in Table. 2. The extract used to carry out antimicrobial screening in vitro on *Staphylococcus aureus, Pseudomonas aeruginosa, Escheria coli, Klebsilla pneumonia, Proteus vulgaris, Streptococcus mutans* indicated significant inhibitory activity against the susceptible organisms. The activities are expressed as resistant, if the *zone of inhibition was less than 7mm,intermediate (8-10mm) and sensitive if more than 11mm* (Assam et al.,2010)

Table.2. Measurement of antibacterial activity of flower and rhizome of extract of Alli (*Nymphea nouchali Burm.f*)

Inhibition zones(mm)					
	Gram (-ve) pathogenic bacteria			Gram(+ve)pathogenic bacteria	
Sample code	Klebsilla sp. MTCC 530	E. coli MTCC 1671	P.vulgaris MTCC 426	S.aureus MTCC 916	S.mutans MTCC 916
AC	8 mm	13 mm	11 mm	14 mm	10 mm
PC	13 mm	11 mm	16 mm	13 mm	13 mm

AC: Alli Chooranam, PC: Positive control

Figures showing zone of inhibition against various pathogens by N.nouchali extract.

Fig.1. *E.coli*

Fig.2.*Staphylococcus aureus*

Fig.3. *Streptococcus mutans*

Fig.4. *Proteus vulgaris*

Fig.5. *Klebsiella pneumoniae*

Nymphea nouchali Burm.f extract found to be effective with concentration of (1mg/ml) against *S.aureus, E.coli, P.vulgaris, S.mutans & Klebsilla sp* suppressing their growth with inhibition zones of 14mm,13mm,11mm,10mm,8mm respectively. High zone of inhibition against *Escheria coli & Staphylococcus aureus over control. Less activity against Klebsilla species.*

Conclusion

The present study suggested that N.nouchali which confirmed to be potentially effective for bacterial infections especially caused by *E.coli & S.mutans* can be used as an alternative therapy for infectious diseases. Phytochemical analysis showed that the *Nymphea nouchali Burm.f* plant extract contains a mixture of phytochemicals as carbohydrates, Alkaloids, Tannins & Flavonoids as secondary metabolites with potential biological activities.

Acknowledgements

The authors would like to acknowledge Biomeitez Research & Development Pvt Ltd for providing us with the necessary lab facilities.

References:

1. Ala et al, 2010,Monitoring and surveillance of chronic non-communicable diseases:progress and capacity in high burden countries, The Lancet,376(9755),P 1861
2. Laddha AP,Kulkarni YA, 2019,Tannins and vascular complications of Diabetes:An update,Pubmed,
3. Murni et al, 2017, Antioxidant and Antidiabetic Effects of Flavonoids: A Structure-Activity Relationship Based Study, BioMed Research International
4. Murugesa Muthaliyar KS, 1936,Gunapadam (MutharPagam-Mooligai Vaguppu), Directorate of Indian Medicine and Homeopathy,Chennai ,2nd edition,43
5. Sadiq et al, 2012,Phytochemistry and antimicrobial activities of Cassia occidentalis used for herbal remedies,Journal of Chemical Engineering
6. Somnath Bhowmik,B.K Data,A.K.Saha, 2013,Ethno medicinal and phytochemical screening of some hydrophytes and marsh plants of Tripura,India,World applied sciences journal