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Screening of Phytochemical Constituents of *Nymphaea Caerulea* savigny. An Aquatic Plant Resource for Drug Development

K. Siva Prasad* and N. Savithramma

Department of Botany, S. V. University, Thirupati-517502, A. P. India

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Address for Correspondence

Department of Botany, S. V. University, Thirupati-517502, A. P. India
E-mail: koyasiva1985@gmail.com

ABSTRACT

Objectives: *Nymphaea caerulea* Savigny. is multipurpose medicinal plant has been mentioned for the treatment of liver disorders in Ayurveda, an ancient system of medicine. Hence the Phytochemical evaluation and estimation of secondary metabolites were carried out.
Methods: Polar and non-polar solvents were used to extract maximum number of compounds from various plant parts of *N. caerulea* preliminary screening analysis (Gibbs, 1974) and quantitative estimation of phytochemicals (Makkar *et al.,* 1993) was carried out.
Results: The results revealed that the plant is a rich source of different secondary metabolites like anthocyanins, anthraqunones, emodins, fatty acids, flavonoids, luecoanthocyanins, glycosides, phenols, coumarins, tannins and triterpenoids. Methanol, ethanol, chloroform and water extracts of leaf and flower are excellent source of phytocostituenpts when compare with rhizome and root.
Conclusion: The findings of the study will be helpful to the pharmacologists and phytochemists for identification of novel potential active compounds.

Keywords: Aquatic plant, Secondary metabolites, *Nymphaea caerulea*, Aqueous extract, Methanol extract, Ethanol extract and Chloroform extract.

INTRODUCTION

The world is looking for new drugs to manage various diseases. All Ayurvada, Unani, Homeopathy and Siddha drugs are depending on herbals. Ayurveda is an ancient health care system and is practiced widely in India and other countries[1]. Roughly 20% of the plants found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced in to the market are obtained from natural or semi-synthetic resources[2].

The investigation of phytochemical compounds like alkaloids, flavonoids, phenols, saponins, tannins, glycosides, terpenoids, coumarins etc. are highly essential to meet the pharmaceutical demand.

In India ten species of *Nymphaea,* both wild (*N. alba*, *N. candida, N. noucholi, N. pubescens, N. rubra and N. tetragona*) and cultivated (Fig.1 b) (*N. caeruleae, N. marliacea, N. micrantha* and *N. alba* var *rubra*), are reported[3]. *Nymphaea* is globally distributed, cultivated, aquatic and Ayurvedic herb with perennial rhizomes or rootstocks anchored with mud with floating or submerged leaves and solitary and showy flowers[4]. It is used in Ayurvedic medicine mainly the flowers are used to wine dyspepsia, enteritis, diarrhoea, urinary problems, fevers and heart palpitations[5]. The leaves, roots, and flowers have a wide range of pharmacological activities and are used for diabetes, eruptive fevers and liver disorders[6]. Number of medicinal plants *Thespesia populnea* and *Tridax procumbens*[7], underutilized species of Cyperaceae[8]. *Dysophylla myosuroides* and *Talinum cuneifolium*[9] were evaluated for phytochemical screening. Though the plant is used in conventionally to cure several diseases, reports on evaluation of phytochemical constituents in *N. caerulea* (Fig.1 a) is not carried so far therefore, the present work is undertaken to screen and estimate the plant for secondary metabolites present in the root, rhizome, leaf and flower of *N. caerulea* as secondary metabolites present in plants are responsible for curing diseases.

MATERIALS AND METHODS

Roots, rhizomes, leaves and flowers (Fig.2 b, c, e and f) were collected from palakonda hills near palakondaraya temple, kadapa District. Washed the *N. caerulea* with tap water and was allowed to dry under shade. The dried roots, rhizomes, leaves and flowers were made in to fine powders separately. These powders were extracted with water, ethanol and chloroform; then the preliminary photochemical tests were carried out for the detection of secondary metabolites.

1) Water extraction

10 g of air dried powder was added to 100 ml of distilled water and boiled for 2 h. The supernatant was collected and same procedure was repeated twice. The collected supernatant at an interval of every 2 h were pooled together and concentrated to make the final volume into one-fourth of the original volume. It was then autoclaved at 121^0C and 15 lbs pressure and stored at 4^0C.[10]

2) Chloroform, Ethanol and Methanol extraction

Dried roots, rhizomes, leaves and flowers each case, powdered air-dried plant materials were extracted with Chloroform, Ethanol and Methanol separately. The crude chloroform extract was prepared by maceration of plant materials (100 g each) with (300 ml) of each solvent[11]; the extracts were filtered and stored in separate bottles.

Phytochemical screening

The condensed extracts were used for preliminary screening of phytochemicals such as flavonoids[12], steroids[13], terpenoids[14], tannins[15], glycosides[16], saponins[14], alkaloids[13], phenols[13], quinones[17], lignin[13], coumarins[18], leucoanthocyanins[15] and emodins[15].

Quantitative analysis of secondary metabolites

The rhizome, root, leaf and flower of *Nymphaea caerulea* were ground to pass through a sieve of 1 mm diameter. Tannins extraction was done using 400 mg ground

sample in conical flask with 40 ml diethyl ether containing 1 per cent acetic acid (v/v) and mixed to remove the pigment material and supernatant was carefully discarded after 5 min., 20 ml of 70 per cent aqueous acetone was added, sealed the flask with cotton plug covering with aluminum foil and kept in electrical shaker for 2 hrs for extraction. Then it was filtered through Whatman No.1 filter paper and sample was kept in refrigerator at 4^0 C until analysis. Then the estimation of phenols and tannins[19], condensed tannins [20] and flavonoids [21] were determined.

RESULTS AND DISCUSSION

The phytochemical screening of various parts of *N. caerulea* showed that they are rich in phenols, flavonoids, saponins, anthraquinones and anthocyanins present in all plant parts but leaves and flowers are showing all phytochemicals in methanol, ethanol and chloroform extractions. Leaves and flowers have more number of secondary metabolites than the rhizome and roots (Table-1). Triterpenoides, alkaloids are absent in root whereas leucoanthocyanins, anthraquinones, triterpenoides and alkaloids are absent in rhizome.

Alkaloids are found only in aqueous extract of flower and leaf. These are produced by large variety of organisms including bacteria, fungi, plants and animals; and are part of group of natural products; some alkaloids have a bitter taste while many to toxic to other organisms [22]. Anthocyanins are present in flowers, leaves and roots extracts of aqueous, chloroform, ethanol and methanol and absent in flowers, leaves, roots and rhizomes extracts of chloroform; and rhizome extracts of aqueous, ethanol, and methanol. Anthocyanins may help the human immune system to work more efficiently to protect against viral infections. It little bit more complex, specific types of anthocyanins may have a direct effect in decreasing influenza viruses' infectivity by decreasing the ability of the virus itself to get into the human cell or to be related from infected cells or by having a viricide effect[22]. Anthraquinones are present in flower and leaf extract of aqueous; root extract of ethanol and flower, leaf and root extracts of methanol. These are absent in root and rhizome extracts of aqueous and flower, leaf, root and rhizome extracts of chloroform; flower, leaf and rhizome extract of ethanol and rhizome extracts of methanol. Anthraquinones are used for stomach-ache and in the treatment of diarrhoea[24] and these are an important chemical raw material and organic intermediates that are broadly applied in the field of dyestuff, papermaking, medicines and agricultural chemicals etc[25].

Coumarins are present only in flowers and leaves in all extracts. Various studies have been demonstrated that coumarins are potential antioxidants and their antioxidant activities are due to their ability to scavenge free radicals and to chelate metal ions[26]. Emodins are found in flower and leaf extracts of aqueous and methanol**.** Emodins are one of the natural anticancer drugs, an active ingredient of the traditional Chinese medicine-rhubrb[27]. Flavonoids are presents in flower, leaf, root and rhizome extracts of aqueous, and absent in flower, root and rhizome extracts of chloroform, root and rhizome extract of ethanol and methanol. Flavonoids are a group of poly phenolic compounds influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes and also act as anti-inflammatory agents[28], the flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process[29, 30]. They also inhibit microbes which are resistance to

antibiotics[31], flavonoids are free radical scarvengers, super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anti-cancer activity[32], as an antioxidants, flavonoids provide anti-inflammatory activity[33] .

Glycosides are present in flower and leaf extracts of aqueous, chloroform, ethanol and methanol; root extract of ethanol, rhizome extract of aqueous and ethanol. These are absent in root extract of aqueous, chloroform and methanol; rhizome extract of chloroform and methanol. Glycosides compounds are containing a carbohydrate and non-carbohydrates residues (moiety) in the same molecule. The carbohydrate moiety is attached an acetyl linkage carbon-I to the non-carbohydrate residue (aglycone). They all contain steroid as aglycone component in combination with sugar molecules. They are important in medicine because of their action on heart and are used in cardiac insufficiency[34]. Leucoanthocyanins are present in flower and rhizome extracts of aqueous and ethanol; leaf and root extracts of methanol and absent in flower, leaf, root and rhizome extracts of chloroform; root and rhizome extracts of aqueous and ethanol, and flower and rhizome extracts of methanol. Leucoanthocyanins are occupying an important position among the water soluble organic compounds. They have been implicated as being responsible for the astringent taste of unripe fruit they are responsible for the chill haze that develops in beer and for the browning of white wine. They influence the storage stability of wine and juice[35]. Steroids are present in flower and leaf extracts of aqueous, ethanol and methanol; flower extract of chloroform, rhizome extracts of aqueous, chloroform, ethanol and methanol and absent in leaf extract of chloroform, root extract of aqueous, chloroform, ethanol and methanol. It should be noted that steroids are of importance and of interest in pharmacy due to their relationship with sex hormones[36]. Phenols are present in flower and leaf extracts of aqueous, ethanol and methanol, leaf extract of chloroform, root and rhizome extracts of aqueous and absent in flower extract of chloroform, root and rhizome extracts of chloroform, ethanol and methanol. Bioactive polyphenols have attracted special attention because they can protect the human body from the oxidative stress which may cause many diseases, including cancer, cardiovascular problems and ageing[37]. Many of the phenols have shown to contain higher levels of anti oxidants activities[38,39], have reported that plant phenols in red wine exerted cardio protective effect.

Saponins are found in flower, leaf, root and rhizome extracts of aqueous; flower, leaf and rhizome extracts of chloroform; flower and rhizome extracts of ethanol, leaf and root extracts of methanol and absent in root extract of chloroform and ethanol; leaf extract of ethanol, flower and rhizome extracts of methanol. Saponins are used as detergents, pesticides and molluscicides, in addition to their industrial application as foaming and surface active agents and also have beneficial health effects[40]. Tannins are present in flower, leaf, root and rhizome extracts of aqueous; leaves extract of chloroform; flower and leaf extracts of ethanol and methanol and absent in flower, root and rhizome extracts of chloroform, root and rhizome extract of ethanol and methanol. Tannins contribute property of astringency i.e. fasten the healing of wound and inflamed mucous membrane and have received considerable attention in the fields of nutrition, health and anti inflammatory properties[41]. Tannins are complex moieties produced by majority of plants as protective substances; they possess wide pharmacological activities and have been used since past as tanning agents and they posses astringent, anti diarrhoeal and

anti-inflammatory activities[42]. Triterpenoids are present in flower and leaf extracts of aqueous, chloroform and methanol. Triterpenoids are absent in flower and leaf extracts of ethanol, and root and rhizome extracts of aqueous, chloroform, ethanol and methanol. Triterpenoids are attributed for analgesic and anti-inflammatory activities. The results revealed that the saponins and glycosides are found in all extracts used in present work. Aqueous extract is the best among the solvents used for extraction of secondary metabolites. Flowers and leaves are rich source of phytochemical constants in order.

Based on the screening of secondary metabolites we conducted the quantitative estimation of some important secondary metabolite like flavonoid, phenols and tannins. The highest total flavonoid content was found in flowers (0.02275 mg QE/g (Quercetin Equivalent)/g) followed by leaf (0.012 QE/g) whereas absent in rhizome and root. Flavonoids are reported to possess many useful properties, including anti-inflammatory, antimicrobial, enzyme inhibition, oestrogenic, antiallergic, antioxidant and antitumour activity[43, 44.]

The highest total phenols content was found in leaf (325 mg TAE (Tannic Acid Equivalent)/g) than flower (315 mg TAE/g), rhizome (210 mg TAE/g) and root (1801 mg TAE/g) (Table-2). Similar results have been shown in *Pongamia* which contain higher levels of antioxidant activity[45]. The higher amount of phenols is important in the regulation of plant growth, development and diseases resistance. It can be used as fungicide, pesticides, an antiseptic, disinfectant and in the manufacture of resins, explosives, plastics, detergents and pharmaceutical substances[46, *i*47,]

The highest total tannins cotent was found in the leaf (90.156 mg TAE/g) and followed by flower (0.11225 mg TAE/g). The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins. Apart from these tannins contribute the property of astringent activity i.e. faster the healing of wounds and inflamed mucous membrane[48.] Studies have indicated that antioxidants prevent the onset of degenerative illness such as certain cancers, cardiovascular and neurodegradative diseases[49].

CONCLUSION

Nymphaea caerulea plants possesses number of secondary metabolites particularly rich source of saponins, glycosides and steroids. The plant widely had used in traditional medicine to combat and cure various ailments. The anti-inflammatory, anti-spasmodic, anti-cancer, anti-microbial, anti-analgesic, anti-oxidants and anti-diuretic activities can be attributed due to the presence of higher levels of steroids, tannins, terpenoids, flavonoids, phenols, emodins and saponins. Exploitation of these pharmacological properties involved further investigation of these active ingredients by implementation of techniques of extraction, purification, separation, crystallization and identification.

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Table 1. Qualitative analysis of phytochemical constituents of various parts of *Nymphaea caeruleae.*

S. No	Phytochemical constituents	Aqueous				Chloroform				Ethanol				Methanol			
		F	L	R	Rh	F	L	R	Rh	F	L	R	Rh	F	L	R	Rh
1.	Alkaloids	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	Anthocyanins	+	+	+	-	-	-	-	-	+	+	+	-	+	+	+	-
3.	Anthroquinones	+	+	-	-	-	-	-	-	-	-	+	-	+	+	+	-
4.	Coumarins	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
5.	Emodins	+	+	+	-	-	+	-	-	-	-	-	-	+	+	-	+
6.	Flavonoids	+	+	+	+	-	+	-	-	+	+	-	-	+	+	-	-
7.	Glycosids	+	+	-	+	+	+	-	-	+	+	+	+	+	+	-	-
8.	Leucoanthocyanins	+	+	-	-	-	-		-	+	+	-	-	-	+	+	-
9.	Steroides	+	+	-	+	+	-	-	-	+	+	-	+	+	+	-	+
10.	Phenols	+	+	+	+	-	+	-	-	+	+	-	-	+	+	-	-
11.	Saponins	+	+	+	+	+	+	-	+	+	-	-	+	-	+	+	-
12.	Tannins	+	+	+	+	-	+	-	-	+	+	-	-	+	+	-	-
13.	Triterpenoids	+	+	-	-	+	+	-	-	-	-	-	-	+	+	-	-

Note: '+' indicates presence, '_ 'indicates absence
(F) Flower, L) Leaf, R) Root and Rh) Rhizome)

Table 2. Quantitative estimation of secondary metabolites

S NO.	*N. caerulea*	Flavonoids Mg QE/g	Phenols mg TAE/g	Tannins mg TAE/g
1	Flower	0.02275	315	011225
2	Leaf	0.012	325	0.156
3	Rhizome	-	210	-
4	Root	-	180	-

'_ 'indicates not found/nil

Fig. 1 a) Author with plant b) Natural habitata of *N. caerulea*

Fig. 2 Various parts of *N. caerulea*
a) Plant b) Roots c) Rhizome d) T.S. of Rhizome e) Leaf f) Flower