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# SCREENING OF *NYMPHAEA TETRAGONA* GEORGI, A TRADITIONAL TAXON FOR BIOACTIVE METABOLITES

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**ABSTRACT** Bioactive metabolites or phytochemical constituents of medicinal plants performing a crucial role in drug industry as well as in identification of elite germplasm Globally distributed *Nymphaea tetragona* Georgi, an aquatic and Ayurvedic herb with perennial rhizomes and white flowers. Studies on phytochemical evoluation for validation in aquatic plants particularly in *Nymphaea* sps. is scanty. Hence the present work is aimed to screen for the analysis of qualitative and quantitative bioactive metabolites present in various parts of the *N. tetragona* for potential applications. Polar and non-polar solvents were used to extract maximum no of compounds from plant parts. The results revealed that the leaf and flower are the rich source of different phytochemicals like anthocyanins, anthraquinones, emodins, fatty acids, flavonoids, luecoanthocyanins, glycosides, phenols, coumarins, tannins and triterpenoids when compare with stem and root. The findings of the study will be helpful to the pharmacologists and phytochemists for identification of an effective and novel phytocompounds.

**KEYWORDS :** aquatic plant, bioactive metabolites, *Nymphaea tetragona*, aqueous extract, methanol extract, ethanol extract and chloroform extract.

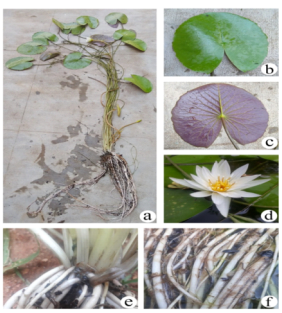
## INTRODUCTION

In many spiritual traditions, lotus flowers are sacred as an offering to the divine. Although the lotus is commonly seen in Buddhist art and literature, it has been a religious symbol in Hindu culture much before the onset of Buddhism. Lotus flowers are divided in to two genera like *Nelumbo* and *Nymphaea. Nymphaea* have many wild species (*N. alba*, *N. candida, N. nouchali, N. pubescens, N. rubra and N. tetragona*) and cultivated (*N. caeruleae, N. marliacea, N. micrantha* and *N. alba*)[1]. The flowers are used in Ayurvedic medicine in wine, dyspepsia, enteritis, diarrhoea, urinary problems, fevers and heart palpitations. Phytochemicals which are naturally occurring in the medicinal plants for defense mechanism and protect from various diseases. Phytochemicals mainly secondary constituents like terpenoids, alkaloids and phenolic compounds were observed in the flowers of *N. caerulea*[2]. Through the plant is used conventionally to cure several diseases, reports on evaluation of bioactive metabolites in *N. tetragona* is not carried out so far. Therefore the present study is undertaken to screen the plant for bioactive metabolites present in the root, rhizome, leaf and flower of *N. tetragona* as secondary metabolites present in plants are responsible for curing number of diseases and the plant had been using to treat several affections by our ancient people.

## MATERIAL AND METHODS

flowers, leaves, rhizomes and Roots, (Fig.1 d b, c, e and f,) of *Nymphaea tetragona* (Fig.1. a) were collected from stacked water near Bokkasenugutta, Sibyala, Rayachoty Mandal, Kadapa District of Andhra Pradesh and washed the parts with tap water and allowed to dry under shade. The dried roots, rhizomes, leaves and flowers were made in to fine powders and stored separately. These powders were primarily extracted separately with water, ethanol, methanol and chloroform; then the preliminary tests were carried out for the detection of primary and secondary metabolites present in these plant parts.

**Fig.1. a) Plant b) Leaf abaxial c) Leaf adaxial d) Flower e) Rhizome and f) Roots,**




### 1. Water extraction

10 g of shade dried powders of plants were added to 100 ml of distilled water and boiled for 2 h. The supernatant were collected and same procedures were repeated twice. The collected supernatants 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$^0$C and 15 lbs pressure and stored in separate bottles at 4$^0$C.[3]

### 2. Chloroform, Ethanol and Methanol extraction

Air dried powders of roots, rhizome, leaves and flowers were extracted with chloroform, ethanol and methanol separately. The crude extracts were prepared by maceration of plant materials (100 g each) with (300 ml) of each solvent[4]. The extracts were filtered and stored in separate bottles.

### Screening of Bioactive compounds

The condensed extracts were used for preliminary screening of primary and secondary metabolites such as proteins[5], starch[6], sugar[7], flavonoides[8], phenols, alkaloids, lignin and steroids[9], saponins and Terpenoids[10], glycosides[11], quinones[12],coumarins[13], tannins, leucoanthocyanins and emodins[14].

## RESULTS AND DISCUSSION

Qualitative and quantitative analysis of the *N. tetragona* revealed that the presence of bioactive metabolites like alkaloids, anthocyanins, anthraquinones, coumarins, emodins, flavonoids, glycosides, leucoanthocyanins, phenols, starch, steroids, sugars, saponins, tannins and terpenoids in flower, leaves, roots and rhizomes as shown in Table 1 and 2. Similar result was observed in the flower, leaves, roots and rhizomes of *N. caerulea*[15].

The primary metabolites like starch, sugars and proteins are also identified from four parts of the plant. The primary metabolites are carbon compounds which are essential to life. All organisms will use primary metabolites as building blocks of the cells and exploit their rich supply of potential energy to maintain life. The rhizome of *N. tetragona* is rich in protein (2.3 gm/dwt) when compare to other plants (Table-2). The presence of higher protein level in plant parts indicates towards possible increase in food value and protein based bioactive compounds could also be isolated in future. Proteins are vital for growth and repair of cells, this may be the reason most of the tribal people store the rhizome, they eat cooked rhizomes. Starch is a nutrient polysaccharides found in rhizome at higher level when compared to other organs of *N. tetragona* (Table-2). These are omnipresence contain storage organs like tubers, seeds and stems are particularly abundant in them[16]. Leaves and flowers of *N.tetragona* also source of starch. But there is no indication in chloroform and methanol extracts.

Sugars found in all extracts of leaves whereas aqueous and ethanolic extracts the flowers contain the sugars and absent in aqueous and chloroform extract (Table-1).The four extracts of roots and rhizomes indicate the absence of sugars. The sugar are help in translocation of food in phloem and protects the plants from desiccation[17]. Sugars are found at higher levels in flowers followed by leaves, rhizomes and roots. The reason could be that the flowers may contain sugars in the form of honey. The phenols are appeared in all plants of aqueous extract and leaves of chloroform, ethanol and methanolic extracts of flowers. The leaves showed maximum levels of phenols when compare with flowers, rhizome and root. The high amounts of phenols are important in regulation of growth, development and disease resistance and plant phenols have anti oxidant and cardio protective activities[18]. They are also used as fungicides, antiseptic disinfectant and in manufacturing of resins.

The aquatic extract contains maximum levels of alkaloids. These are the most diverse group of secondary metabolites present in living organisms with all arrays of structural types in biosynthetic pathways and various pharmacological activities. The anthocyanins found which are in the leaves, flowers and roots of *N. teteagona* and absent in rhizomes. Anthocyanins possess viricide effect. Not only decreasing influenza viral infection but also prevent the entry of virus to human cell[19]. The leaf and flower extracts of aqueous ethanol, methanol and chloroform showed the coumarin, which are potential anti oxidents by scavenging free radicals and chealating metal ions [20]. Emodins found in the flowers of all extracts. Emodins are one of the natural anti cancer drugs Chinese used as anti cancer drugs in the traditional medicine[21]. Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extract which may also be used to search for bioactive agents that could be used in the synthesis of very vital drugs[22]. Flavonoids are present in flower, leaf, root and rhizome extracts of aqueous and absent in chloroform extract, root and rhizome extracts of ethanol and methanol. As flavonoids are water soluble and potent asfree radical scavengers, super antioxidants, prevent oxidative cell damage and have strong anti-cancer activity[23].

**Table 1. Qualitative analysis of bioactive metabolites of various parts of *N. tetragona***

| 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. | Starch | + | + | - | - | - | + | - | - | + | + | - | - | - | + | - | + |
| 2. | Sugers | + | + | - | - | - | + | - | - | + | + | - | - | - | + | - | + |
| 3. | Proteins | + | + | - | + | - | + | - | - | + | + | - | - | + | + | + | + |
| 4. | Alkaloids | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5. | Anthocyanins | + | + | + | - | - | - | - | - | + | + | - | - | + | + | - | - |
| 6. | Anthraquinones | + | + | + | - | - | - | - | - | - | + | + | - | + | + | - | - |
| 7. | Coumarins | + | + | - | - | + | + | - | - | + | + | - | - | + | + | - | - |
| 8. | Emodins | + | + | - | - | - | - | - | - | - | + | - | - | + | - | - | - |
| 9. | Flavonoids | + | + | - | + | - | + | - | - | + | + | + | - | + | + | - | - |
| 10. | Glycosides | - | - | - | - | - | + | - | - | - | - | - | - | + | + | - | - |
| 11. | Leucoanthocyanins | + | + | - | - | - | - | | - | - | - | - | - | - | - | - | - |
| 12. | Steroids | + | + | - | + | + | - | - | - | + | + | - | + | + | + | - | + |
| 13. | Phenols | + | + | + | + | - | + | - | - | + | + | - | - | + | + | - | - |
| 14. | Saponins | + | + | + | + | + | - | - | - | + | - | - | - | + | + | + | - |
| 15. | Tannins | + | + | - | + | - | + | - | - | + | + | - | + | + | + | - | - |
| 16. | Triterpenoids | + | + | - | - | - | - | - | - | - | + | - | - | + | - | - | - |

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

**Table 2. Quantitative analysis of bioactive metabolites of various parts of *N. tetragona***

| S. No | N. tetragona | Secondary bioactive metabolites | | | Primary bioactive metabolites | | |
|---|---|---|---|---|---|---|---|
| | | Flavonoids mg/g dwt | Phenols mg/g dwt | Tannins mg/g dwt | Starch mg/g dwt | Sugar mg/g dwt | Proteins mg/g dwt |
| 1 | **Flower** | 0.026 ± 0.002 | 280 ± 2.943 | 0.13 ± 0.008 | 3.7 ± 0.061 | 6.9 ± 1.632 | 1.8 ± 0.163 |
| 2 | **Leaf** | 0.0127 ± 0.001 | 285 ± 4.198 | 0.16 ± 0.012 | 3.7 ± 0.526 | 4.0 ± 0.816 | 1.8 ± 0.081 |
| 3 | **Rhizome** | - | 210 ± 3.681 | - | 4.1± 0.552 | 3.4 ± 1.632 | 2.3 ± 0.205 |
| 4 | **Root** | - | 180 ± 2.943 | - | 33.7 ± 0.526 | 2.3 ± 1.632 | 1.2 ± 0.124 |

**Note:** '_ 'indicates absence and ± indicates stranded error

Glycosides are present in flower and leaf extracts of aqueous, chloroform, ethanol and methanol and root extract of ethanol, rhizome extracts of aqueous and ethanol. They are absent in root extracts of aqueous, chloroform and methanol, rhizome extracts of chloroform methanol. Glycosides compounds are containing a carbohydrate and non-carbohydrate residues (moiety) in the same molecule. The carbohydrate moiety is attached to 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[24].

Leucoanthocyanins are present in flower and leaf extracts of aqueous only and absent in chloroform, ethanol and methanol of flower, leaf, root and rhizome extracts. 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 also 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[25]. Steroids are present in flower and leaf extracts of aqueous, ethanol and methanol; flower extract of chloroform, stem extract of aqueous, chloroform, ethanol and methanol and absent in leaf extract of chloroform, root extract of aqueous, chloroform, ethanol and methanol. Steroids are important in pharmacy as they related to sex hormones.[26].

Saponins are found in flowers, leaves, roots and rhizome extracts of aqueous; flowers, leaves and rhizome extracts of chloroform; flower and rhizome extracts of ethanol, leaves and roots extracts of methanol and absent in root extract of chloroform and ethanol; leaf extracts of ethanol, flower and rhizome extracts of methanol**.** The saponins are used as detergents, pesticides and moluscides along with their industrial applications in foaming and surface active agents. They are involved in controlling of onset of labour pains along with oxytocin[27].

Tannins are present in flowers, leaves, roots and rhizome extracts of aqueous; leaf extract of chloroform; flower and leaf extracts of ethanol and methanol and absents in flower, root and rhizome extracts of chloroform, root and rhizome extract of ethanol and methanol. Tannins contribution in wound healing and reduced the inflammation of mucus membranes. They possess astringent, anti diarrhoeal and wide pharmaceutical properties[28].

Triterpenoids are present in flower and leaf extracts of aqueous, chloroform and methanol. Triterpenoids are absent in flower and leaf extract of ethanol, 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 constituents in order.

Based on the screening of bioactive metabolites among the flavonoids, phenols, tannins, starch, sugar and proteins highest content of flavonoids were found in flowers (0.026± 0.002 mg/g dwt) followed by

leaves (0.0127± 0.001 mg/g dwt). Flavonoids are reported to possess many useful properties, including anti-inflammatory, antimicrobial, enzyme inhibition, oestrogenic, antiallergic, antioxidant and antitumour activity[29,30].

The present study is evident that the *N. tetragona* posses number of medicinal properties, that might be the reason the taxon has been using in traditional events since ancient times. *N. tetragona* is proved that the plant parts are rich in bioactive metabolites like other *Nymphaea* species like *N .caerulea*[31]. *N. pubescens*[32]. *N. rubra*[33] and can be utilise in potential pharmacological applications.

## CONCLUSION.

*N. tetragona* plant appears to be rich in primary and secondary metabolites particularly rich source of flavonoids, saponins, glycosides and steroids. 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 to their high amount of steroids, tannins, terpenoids, flavonoids, phenols, emodins and saponins. Exploitation of these pharmacological properties involves further investigation of these active ingredients by implementation techniques of extraction, purification, separation, crystallization and identification.

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