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Medicinal values of aquatic plant genus *Nymphoides* grown in Asia: A review

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ABSTRACT

Nymphoides is an aquatic genus consisting about 50 species, of which few were accepted as traditional medicinal plants in Asia. The literature review revealed that *Nymphoides* species are widely used in Ayurvedic medicine as a popular drug, *i.e.* Tagara. They are also utilized by the traditional local healers of different Asian countries to treat various diseases, like convulsion, jaundice, fever, headache, *etc.* According to the *in vivo* and *in vitro* pharmacological studies, *Nymphoides* species have been claimed to possess major biological activities like anticonvulsant, antioxidant, hepatoprotective, cytotoxic, antitumor, *etc.* Biochemical profiling of different aquatic plants of this genus revealed the presence of some important phytochemicals as polyphenolic component, flavonoids, triterpenes, carbohydrates, glycosides, *etc.* Several valuable bioactive compounds including ephedrine, coumarin, secoiridoid glucosides, methyl quercetin, ferulic acid, foliamenthic acid, *etc.* were also known to be isolated and identified from different *Nymphoides* species. The aim of this review is to analyze the published report based on the medicinal values of different Asian *Nymphoides* species, to provide the updated information about the ethnomedicinal, pharmacological as well as the phytochemical properties for the first time.

1. Introduction

Aquatic plants have many unique biological features and are potential for its agricultural, nutraceutical and medicinal importances. Many plant species under aquatic origin were reported to have valuable folklore utilization in traditional medicine[1]. The genus *Nymphoides* is one of the most ubiquitous floating-leaved groups, belonging to Menyanthaceae family of aquatic and wetland plants and it has about 50 species globally. The name *Nymphoides* refers to be similar to the water lily *Nymphaea*[2]. The plant species under *Nymphoides* consist of roots under water and floating leaves holding the small flowers above the water surface. This aquatic genus is extensively distributed worldwide in the tropical and temperate regions[3]. Among

them, few species of *Nymphoides* namely *Nymphoides indica* (*N. indica*), *Nymphoides peltata* (*N. peltata*), *Nymphoides hydrophylla* (*N. hydrophylla*) and *Nymphoides macrospermum* (*N. macrospermum*) are available in the southern region of the Asian continent. These species are known to be involved in a multi medicinal usage locally. According to ethnobotanical reports, these aquatic herbs are traditionally used for the treatment of injuries, snake bites, jaundice, dysentery, convulsion *etc.* as well as constituent of a well-known ayurvedic drug formulation, *e.g.* Tagara[4,5].

The phytochemical constituents of the *Nymphoides* species are found to be comprised of various lipophilic compounds, flavonoids, β -sitosterol, salicylic acid, tannic acids *etc.*[6,7]. Literature reviews

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revealed different pharmacological studies including antimicrobial, antidiabetic, antiprotozoal, antioxidant, cytotoxic, anti-proliferative etc. on this aquatic species. In Asia, many of the indigenous aquatic plants of the genus *Nymphoides* are extensively used in traditional medicine, and various research works were done to investigate their bioactivity and their phytochemical constituent till now. The present article reviews those medicinal values of this aquatic genus *Nymphoides* to highlight the significance and the importance of such floating plant species as ethnomedicines.

2. *Nymphoides* species in folklore practice

According to the ethnobotanical study, there is a huge multicultural history which was documented in Asia to cure different diseases by using the herbal extracts of the *Nymphoides* species..

N. indica (Linn.) O. Kuntze, the most well-known species for the local healers of Bangladesh, India and Nepal, which is provided as a general tonic in replacement of chiretta (*Swertia chirayita*) for the symptomatic treatment and also for the management of jaundice, dysentery and fever[8–10]. In South Orissa, *N. indica* is known to cure a bilious headache, scabies, rheumatism by the external application of the raw leaf paste and also the sesame oil soaked whole dried plant[11]. The juice obtained from the leaves and roots of *N. indica* was used as a component of an oral herbal formulation with other plant extracts, i.e. *Achyranthes aspera* and *Piper nigrum* prepared by the Bagdi tribal healer of Rajbari District, Bangladesh for the treatment of common ailments, such as tonsillitis to relief pain[12].

N. hydrophylla (Lour.) O. Kuntze is another common species of *Nymphoides* native in Bangladesh, India, Bhutan, and Cambodia. The plant is frequently used as a substitute of chiretta (*Swertia chirayita*) in the treatment of fever and jaundice. Dried powder of stalks and leaves are mixed with different herbal oil formulation and applied in various skin ulcers and insect bite. The fresh leaf paste is considered as an antidote for scorpion sting and snake bite. The decoction of stalk and leaf is used externally as an antiseptic and also used as a cleanser for the management of parasitic skin infections. The raw fluid of the leaf is applied superficially in various ophthalmic diseases. Seed powder of the plant is known to be orally administered with honey as an anthelmintic drug[11,13].

N. peltata (Gmel.) O. Kuntze is also the popular floating plant native to the temperate regions of Europe, Asia and the Mediterranean region. In India, the plant is considered as medicinally important mostly in Kashmir wetlands, where it is therapeutically administered as an anthelmintic, diuretic and febrifuge and also reported to be used in the treatment of burns, fevers, ulcers, snake bites and swellings. The fresh leaves paste is used in the treatment of habitual headache like migraine, migrainous neuralgia, etc. It is also administered during pregnancy as galactagogue to promote lactation[14].

N. macrospermum Vasudevan is the renowned *Nymphoides* species

in Ayurvedic medicine which is used to treat various chronic diseases like epilepsy, anemia, jaundice, tuberculosis, etc. And it is commonly used as a substitute of *Valeriana jatamansi* Jones in the ayurvedic drug Tagara that is known to minimize the severity of convulsions[15,16].

3. Ethnomedicinal formulation of *Nymphoides* species

Nymphoides species are constituent of Tagara which is one of the most reputed ayurvedic drug formulation, it is generally regarded as brain tonic. It is also commonly used in Unani medicine, Asarun (Asarum) and in various pharmaceutical preparations for the treatment of brain and nervous system disorder, like migraine, insomnia, anxiety, epilepsy. *Nymphoides* species are also known to cure anaemia, jaundice and asthmatic conditions. However, the most established source of Tagara is *Valeriana jatamansi* (Valerianaceae), and to some extent, due to their inconvenient sources, i.e. Himalayas and another drug 'Granthika Tagara' from South India consisting of *N. macrospermam*, started being used as Tagara in several therapeutic preparations. Granthika Tagara is a combination of numerous available *Nymphoides* species, like *N. macrospermam*, *N. hydrophylla*, *N. indica*, *Nymphoides aurnaticum* and *Nymphoides parvifolium*, etc. found in different parts of India[17,18].

4. Reported pharmacological activity

4.1. Anti-convulsant activity

Herbal drugs have been invariably used in various traditional medicines for the treatment and management of epilepsy, due to the avoidance of the associated side effects and dependency development which was caused by prolonged use of other prescribed synthetic drugs[19]. Therefore, researchers are now very curious in discovering drugs from the natural sources and studying their pharmacological properties regarding anticonvulsant activities. *N. indica* and *N. macrospermum* showed anticonvulsant activities and found to possess the same mechanism as benzodiazepines, phenobarbitone, felbamate, etc., by inducing the delayed onset and discontinuation of seizures and the rapid recovery without any toxicity report after consumption. *N. indica* is known to be effective against petitmal and grandmal type of epilepsy, which is confirmed by investigating the anticonvulsant effect using aqueous and alcohol extract of their roots and rhizomes by maximum electroshock convulsion and pentylenetetrazole induced convulsion methods in Swiss albino mice. The results indicated that the extracts of *N. indica* at the dose level of 300 mg/kg and 600 mg/kg exhibited significant anticonvulsant activity against both maximum electroshock convulsion induced and pentylenetetrazole induced convulsion models[20]. *N. macrospermum* was also reported to be effective against the petitmal type of epilepsy

and it was found that the alcohol extract of *N. macrospermum* at doses of 500 mg/kg and 750 mg/kg bodyweight, extremely diminished the severity and increased the latency of convulsions in the pentylenetetrazole induced seizure model[21]. Due to these excellent pharmacological properties, *N. indica* and *N. macrospermum* plant species are considered as a probable source of Tagara to establish their use as a substitute for *V. jatamansi*.

4.2. Sedative activity

Antiepileptics are generally consumed with other anxiolytic drugs. As majority of antiepileptic drugs have to be consumed constantly, concomitant administration of other drugs induces the risk of drug interaction. By considering these factors, researchers are now interested in compounds from plant origin for developing anxiolytic drugs with minor side effects. Recently, there are many reports of traditional drugs exhibiting good hypnotic and sedative effects with few side effects. *N. macrospermum* is considered as the potential source of such traditional drugs formulation, e.g. Tagara. It has the antiepileptics properties and was found to provide sedative effect investigated by a significant reduction in locomotor activity in Swiss albino mice which was studied by using an actophotometer. The alcohol extract of *N. macrospermum* up to a dose of 750 mg/kg body weight caused central nervous system depression which was indicated by a reduction in locomotor activity without inducing any toxicity, therefore it is reported that it has no acquired side effect[21,22].

4.3. Antioxidant activity

Medicinal plants have always been recognized as a potential source of natural antioxidants from an ancient period of times. Among *Nymphoides* species, *N. hydrophylla* and *N. indica* are reported to have antioxidant property. *N. indica* is claimed to possess moderate antioxidant activity. Recent study indicated that the ethyl acetate extract of *N. indica* leaf fractions presented better antioxidant potential (IC₅₀ 81 µg/mL) determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity[6]. There are numerous studies for determining the antioxidant activity in terms of its aqueous, ethanolic and methanolic extracts of *N. hydrophylla*. According to the literatures, it can be suggested that the ethanolic extract of *N. hydrophylla* has significant antioxidant activity compared to other extracts. In that study, the ethanolic extract of *N. hydrophylla* was tested by using four types of antioxidant methods, DPPH free radicals scavenging activity, reducing power method, nitric oxide assay and phosphomolybdenum method against standard gallic acid. According to the result, *N. hydrophylla* extract showed effective scavenging abilities against the free radicals by acting as hydrogen donors to scavenge DPPH free radicals with IC₅₀ values of 6.35 µg/mL, by increasing the reductive power resulting the increase in the absorbance of the reaction mixture by the plant extract in a linear manner, and also by scavenging of nitric

oxide radical with IC₅₀ values of 432 µg/mL causing decreased amount of nitrite generation[23]. The comparative study of the ethanolic and aqueous extract of *N. hydrophylla* were performed in another study, where it was found that the ethanolic extract exhibited higher antioxidant activities than the aqueous extract in the anti-lipid peroxidation, reducing power, metal chelation, and 2,2-azino-bis (3-ethylbenthiazoline-6-sulfonic acid) or ABTS radical scavenging assays, whereas the aqueous extract possessed more potent activity in the superoxide and DPPH radical scavenging assays[24]. The crude methanolic extract of *N. hydrophylla* was also examined in another study for possible antioxidant activity, where the total phenolic content in leaves of *N. hydrophylla* extract was found to be 12.5 mg of gallic acid equivalent (GAE)/g of extract indicating the moderate antioxidant activity[6].

4.4. Hepatoprotective activity

Plant extract having antioxidant activities also leads to the inhibition of oxidative damage to a targeted tissue. Considering such antioxidant potential, the *in vivo* hepatoprotective effect of *N. hydrophylla* was measured against the carbon tetrachloride (CCl₄) induced liver injury in albino rats. According to this study, it was demonstrated that the ethanolic whole plant extract of *N. hydrophylla* reduced the increased levels of biochemical parameters, like serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, alkaline phosphatase and bilirubin, etc. As indicated in CCl₄ induced hepatotoxicity and following histopathological studies, it was reported that the ethanolic extract (500 mg/kg) of *N. hydrophylla* produced impressive hepatoprotective activity without any necrosis and sinusoidal dilatations resembling to the standard ‘Silymarin’ group. Simultaneous treatment of ethanolic extract with CCl₄ was found to produce lower hepatic damage in contrast to the animals treated with CCl₄ alone which caused hepatotoxicities, like macro and micro vesicular fatty infiltration, necrosis, sinusoidal dilatation and congestion of central vein etc. It was suggested that the possible mechanism of such hepatoprotective action exhibited by *N. hydrophylla* could be the lipid peroxidation inhibition and free radicals scavenging effects[23].

4.5. Cytotoxic activity

Cytotoxic screening of plants is the preliminary method that seems to be capable of detecting natural anticarcinogenic agents present in crude extracts. According to the literature, the leaf of *N. hydrophylla* was found to be cytotoxic which was investigated by using brine shrimp lethality bioassay, where the result showed that the crude methanolic extract of leaves of *N. hydrophylla* had significant cytotoxic activity with LC₅₀ values found to be 3.28 µg/mL as compared to the standard vincristine sulphate with LC₅₀ value 0.84 µg/mL. Through performing this general bioassay, it can be considered that such valuable species of *Nymphoides* genus can be the potential

source of anticancer compounds[25,26].

4.6. Antitumor activity

Most antitumor drugs are generally encountered through plant-derived compounds or their derivatives. Currently, few *Nymphoides* species are accounted for its antitumor activity. Among them, *N. peltata*, the common wetland plant, exhibited significant antitumor activity in MTT assay by using 10% H₂O-methanol (MeOH) extract of *N. peltata* against the human prostate cancer cell PC3 and the human osteosarcoma cell U2OS with IC₅₀ values of 40.8 µg/mL and 70.7 µg/mL, respectively, where PC3 cells showed more sensitivity to the extract. Based on the phytochemical study, the proposed antitumor effect of *N. peltata* extract is thought to be due to the presence of the four major antitumor constituents, i.e. ephedrine, ephedradine C, 4-hydroxycoumarin and delta-1-dehydrotanshinone[27]. The aqueous extract of *N. indica* was also reported to have higher anti-proliferative activity on HeLa cell line[28].

4.7. Antidiabetic property

Diabetes is a serious complication attributed to several metabolic disorders. A literature review revealed that *N. indica* leaf extract and its isolated constituents possessed mild to moderate antidiabetic property, which was experimented through in vitro antidiabetic assays, i.e. antiglycation assay and α-glucosidase inhibitory activity. Following antiglycation assay, it was observed that the crude extract of *N. indica* presented mild to moderate inhibitory activity at 100 µg/mL (33%), among which 90% methanolic extract exhibited most promising activity. Such antiglycation potential by the tested extracts showed moderate (24%-36%) inhibition of the formation of advanced glycation end products. Considering α-glucosidase inhibitory activity, a mild inhibition was observed for the total extract at 834 µg/mL (13%), whereas the highest inhibition was shown by 90% methanol (31%) and *n*-butanol fractions (25%) at the same concentration. The isolated seco-iridoid glucosides, monoterpenoids, phenolic acids and flavonoids from different fractions of *N. indica* extracts showed moderate activity towards antiglycation and inhibitory potential against the enzyme like α-glucosidase[6].

4.8. Antimicrobial activity

Aquatic medicinal plants have always been considered as potential sources for investigating antimicrobial properties which are intended for the new drug development[29]. The antimicrobial effects of such aquatic *N. indica* species were reported to be carried out through in vitro antimicrobial assay in an integrated screening panel. In this study, it was observed that the *n*-hexane fraction of *N. indica* leaf extract showed the highest levels of antimicrobial and antifungal activities with an IC₅₀ of 19.5 µg/mL against *Staphylococcus aureus* and 32 µg/mL against *Microsporium canis*, and it was claimed that

the isolated lipophilic constituents from that *n*-hexane fraction showed moderate antimicrobial activities among which the isolated compound, azelaic acid was highly active against *Staphylococcus aureus* with an IC₅₀ value of 55.1 µM (11.2 µM for erythromycin). The 90% methanolic fraction of *N. indica* extract was also found to be active (IC₅₀ 36.4 µg/mL) against *Staphylococcus aureus*[6].

4.9. Antiparasitic effect

There are numerous medicinal plants known to be used as potent antiparasitic agent which are still under investigation for the development of novel antiparasitic drug. *Nymphoides* species are reported to have various antiparasitic properties, like antiprotozoal, antimalarial and anthelmintic, etc. An experiment reported that the isolated lipophilic constituents and the flavonoid i.e. 3, 7-Di-O-methyl quercetin-4'-O-β-glucoside from *N. indica* leaf extract showed antiprotozoal activities against *Trypanosoma brucei* (IC₅₀ 8 µM), *Leishmania infantum* (IC₅₀ 32 µM) and *Trypanosoma cruzi* (IC₅₀ 30 µM). Azelaic acid, the lipophilic compound isolated from *N. indica* leaf extract also showed weak antiplasmodial activity against *Plasmodium falciparum* K1[6]. The crude methanolic leaf extracts of *N. hydrophylla* is reported to exhibit significant anthelmintic property against *Pheritima posthuma* earthworm in a dose-dependent manner, where the results were expressed in terms of time for paralysis and time for death of worms. According to the result, the extract exhibited maximum efficacy at a concentration of 80 mg/mL compared to the standard piperazine citrate at dose of 15 mg/mL demonstrating the mild anthelmintic activity of *N. hydrophylla*[25].

5. Phytochemical investigation

5.1. Phytochemical screening

Preliminary phytochemical screening of *Nymphoides* species revealed the presence of many bioactive components, like flavonoids, tannins, saponins, triterpenes, carbohydrates, glycosides, phenolic compounds, phytosterols, volatile oil, coumarins, fixed oils and fats, steroids and sugars from different alcohol and aqueous extracts of *N. hydrophylla*, *N. indica* and *N. macrospermum*. In *N. indica*, the total flavonoid content of the crude extract was found 70.28 mg RUE/g extract, similarly the total phenolic content was 28.88 mg of GAE/g[25]. In *N. hydrophylla*, the total polyphenolic compounds was found (1.110 ± 0.318) mg/g and (1.800 ± 0.350) mg/g in both aqueous and alcohol extract, respectively[6,7,20].

5.2. Identification and isolation of bioactive compound

β-sitosterol (1), betulinic acid (2), salicylic acid (3) and tannic acid (4) were reported for the first time from *N. hydrophylla* (Figure 1). A triterpenoid compound, β-sitosterol was reported to be identified and isolated in the alcohol extract of roots and rhizomes of *N.*

hydrophylla. The percentage yield of the isolated β -sitosterol was found 24% and the melting point was 136 °C-137 °C. However, no structure elucidation of that isolated β -sitosterol was reported in this study[7].

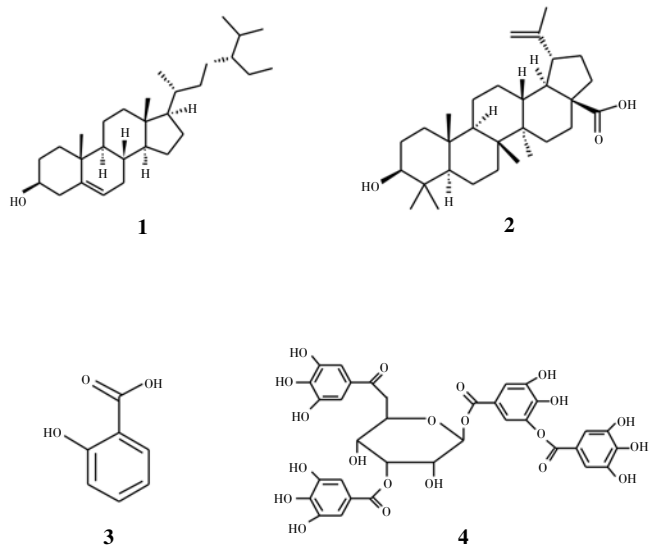


Figure 1. Identified phenolic compounds from *N. hydrophylla*. β -sitosterol (1), betulinic acid (2), salicylic acid (3) and tannic acid (4).

Literature search showed that four major antitumor constituents including ephedrine (1), ephedradine C (2), the organic amines, 4-hydroxy-coumarin (3), the benzopyrone compound and delta-1-dehydrotanshinone (4) were identified from the 10% H₂O-MeOH extract of *N. peltata* (Figure 2) through HPLC and quadruple time-of-flight mass spectrometry analysis combined with the Agilent TCM database, METLIN database, and spectral data of standard compounds.

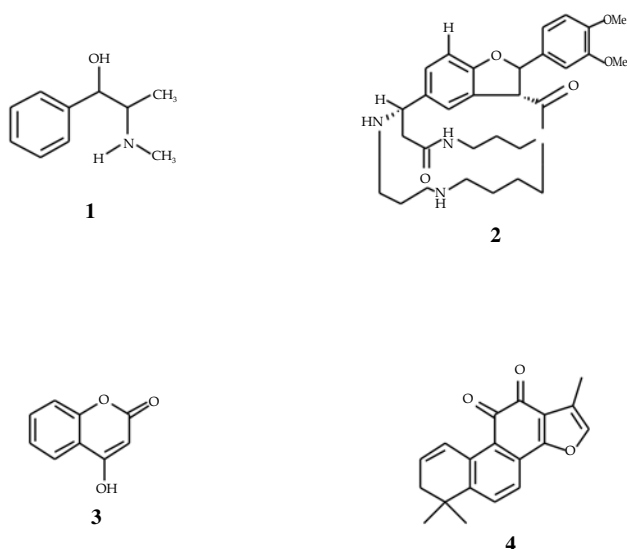


Figure 2. Antitumor constituents detected from *N. peltata*. Ephedrine (1), ephedradine C (2), the organic amines, 4-hydroxy-coumarin (3), benzopyrone compound and delta-1-dehydrotanshinone (4).

According to the literature, these compounds are considered as the major active ingredients that are rarely reported from wetland plant *N. peltata* and other *Nymphaoides* genus[29]. Gibberellins were also reported to be detected from the endophytic fungus *Aspergillus clavatus* isolated from the roots of *N. peltata* (Figure 3). Based upon quantitative assay using gas chromatography-mass spectrometry-selected ion monitoring analysis of the culture filtrate of *Aspergillus clavatus* Y2H0002 strain, three bioactive gibberellins (GAs: GA1, GA3 and GA4) were detected and quantified considering the physiologically active hormones[30].

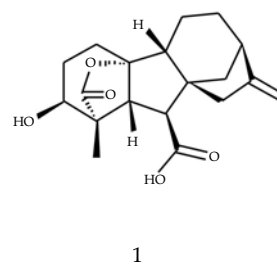


Figure 3. Gibberellins detected from endophyte *A. clavatus* isolated from the *N. peltata*.

Phytochemical investigation of the *n*-hexane fraction leaves of *N. indica* species claimed 5 lipophilic constituents (Figure 4) identified as azelaic acid (1), hexadecanoic acid (2), 4-methyl-heptanedioic acid (3), hexadecanol (4) and stearic acid (5) based on 1H- and 13C-NMR spectral analysis and GC-MS analysis.

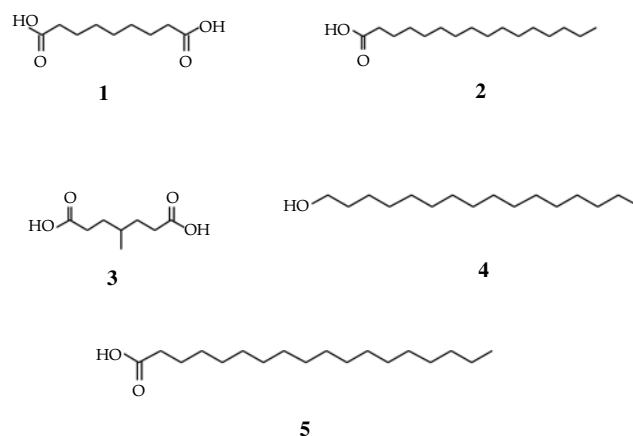


Figure 4. Identified lipophilic constituents from *N. indica*. Azelaic acid (1), hexadecanoic acid (2), 4-methyl-heptanedioic acid (3), hexadecanol (4) and stearic acid (5).

For the isolation of pure compounds, semi-preparative HPLC-DAD-MS system was followed and structure elucidation was done for the remaining compounds (6-15) by 1D- and 2D- NMR spectroscopic methods (Figure 5). Among them, three seco-iridoid glucosides (6–8) and three flavonoids (9-11) were reported to be isolated and

identified as 7-Epiexaltoside (6), 6'',7''-Dihydro-7-epiexaltoside (7), menthiafolin (8) and 3,7-di-O-methylquercetin-4'-O- β -glucoside (9), 3-O-methyl quercetin-7-O- β -glucoside (10) and 3,7-di-O-methyl quercetin (11). Finally, the phenolic coumarin, hydroxycinnamic acid, monoterpenoid and ester were also reported to be isolated and identified as scopoletin (12), ferulic acid (13), 8-hydroxy-2,6-dimethyl-(2E,6E)-octadienoic acid or foliamenthic acid (14) and 6,7-dihydrofoliamenthic acid methyl ester (15) respectively[6].

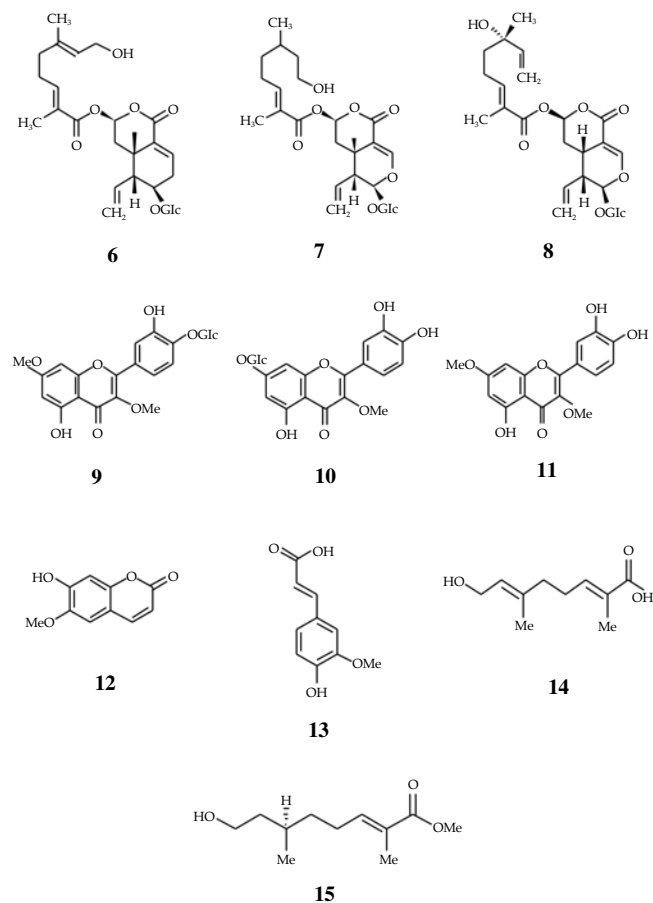


Figure 5. Isolated compounds from *N. indica*.

7-Epiexaltoside (6), 6'',7''-Dihydro-7-epiexaltoside (7), Menthiafolin (8) and 3,7-di-O-methylquercetin-4'-O- β -glucoside (9), 3-O-methyl quercetin-7-O- β -glucoside (10) and 3,7-di-O-methyl quercetin (11). Finally, the phenolic coumarin, hydroxycinnamic acid, monoterpenoid and ester were also reported to be isolated and identified as scopoletin (12), ferulic acid (13), 8-hydroxy-2,6-dimethyl-(2E,6E)-octadienoic acid or foliamenthic acid (14) and 6,7-dihydrofoliamenthic acid methyl ester (15).

6. Adverse effect and safety

There is limited information documented about the risk of *Nymphoides* species. According to the literature, motor neuron impairment was reported for *N. macrospermum* as a side effect of its anticonvulsant activity[21].

7. Conclusion

In Asia, *Nymphoides* species are very popular phytomedicine for the local healers. Huge pharmacological studies have been carried out regarding different species of *Nymphoides*. Among all medicinal properties of *Nymphoides* species, their anticonvulsant property is very important for establishing ayurvedic drug, *i.e.* Tagara. The phytochemical screening and the reported pharmacological activities of different *Nymphoides* species suggested the presence of valuable bioactive compounds. Therefore, extensive research should be necessary in the area of isolation and characterization of the compound from that medicinally renowned plant species of *Nymphoides* for the purpose of new drug development.

Conflict of interest statement

Authors declare that there is no conflict of interest.

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