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# SCREENING OF BIOACTIVE COMPOUNDS IN HYDROPHILA AURICULATA LEAVES BY HPLC, FTIR AND UV-VISIBLE SPECTROSCOPY

Chemistry

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## ABSTRACT

The present study was aims to investigate the Bioactive compounds in *Hydrophila auriculata* leaves using HPLC, FTIR and UV-Visible Spectroscopy. The results showed the presence of bioactive compounds in aqueous extract of *Hydrophila auriculata Vi2 t*annin, saponnin, flavonoids,terpenoids, triterpenoids, carbohydrate, polyphenol were present ,while phlobatannins, steroids, alkaloids, protein, anthroquinone and glycoside were absent. Methanol extract of *Hydrophila auriculata* leaves showed the presence of tannin, saponnin, flavonoids, steroids, alkaloids, terpenoids, triterpenoids, anthroquinone, carbohydrate and polyphenol while phlobatannins, protein and glycoside were absent. Tannin, Saponnin, Flavonoids, Steroids, Alkaloids, Triterpenoids, Polyphenol were present while Phlobatannins, Terpenoids, Carbohydrate, Protein, Anthroquinone and Glycoside were absent in ethanol extract of *Hydrophila auriculata* leaves. Significant quantity of Phenol (204.23±14.28), Flavonoids (125.78±8.75), Alkaloids (68.77±4.80), Tannin (40.32±2.8) and Terpenoids (58.63±4.06) present in *Hydrophila auriculata* leaves Results of the UV spectrum indicates the presence of flavonoids , phenol and its derivatives in the *Hydrophila auriculata* leaves. The results of the FTIR confirmed the presence of alcohol, phenol, Alkenes, Aromatics, Carboxylic acids, Aliphatic amines and Nitro compounds. HPLC profiles of *Hydrophila auriculata* were analysed and seven phenolic compounds namely Kaempferol, P-coumaric Acid, Epicatechin,Tannic acid, Epigallocatechin, Ellagic acid and Naringenin were identified.. This supports the popular use of *Hydrophila auriculata* leaves in preparation of various pharmaceutical formulations for human welfare.

## KEYWORDS

*Hydrophila auriculata* , Phytochemical screening, Phytonutrients, HPLC, UV- Visible spectrum, FTIR.

## INTRODUCTION

India is sitting on a gold mine of well-recorded and traditionally well-practiced knowledge of herbal medicines, therefore any scientific data on such plant derivatives could be of clinical importance. Medicinal plants are the richest bio-resource of drugs of traditional system of medicine, modern medicines, preparation of nanomaterials, nutraceuticals intermediates and chemical entities for synthetic drugs. Medicinal plants are of great importance to the health of individuals and communities They are generally used in traditional medicine for the treatment of many ailments (Nijoku and Ezeibe, 2007).

Many plants contain a variety of phytopharmaceuticals, which have found very important applications in the fields of agriculture, human and veterinary medicine. The use of plants as therapeutic agents in addition to being used as food is age long and there is a great awareness in the use and significance of these medicinal floras (WHO 2002). This has led to intensified efforts on the documentation of medicinal plants (Perumal *et al.,* 2000). Phytochemicals are natural bioactive compounds found in plants that functions along with dietary fibre and nutrients which protect against various diseases. Vegetables, leaves and nuts are the rich sources of phytochemicals help in slow aging and reduce the risk of many diseases including diabetes, heart diseases, infections, cancer etc.( Sudhanshu *et al.,* 2012). Some important phytochemicals are alkaloids, flavonoids, tannins,saponins that posses antifungal, antibacterial, anticancer, anti-inflammatory characteristics (Lingarao and Savithramma, 2011).The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drug for curing of various diseases The knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folk medicines (Mojab *et al.,* 2003). Secondary metabolites are reported to have many biological and therapeutic properties. Pharmacists are interested in these compounds because of their therapeutic performance and low toxicity (Inaya. tullah et al., 2012). On the basis of therapeutic potential of secondary metabolites, The phytochemicals are determined in the *Hydrophila auriculata (Linn)*leaves , which may provide an insight in its use in traditional medicine.

## MATERIALS AND METHODS

### Collection of *Hydrophila auriculata* leaves

The *Hydrophila auriculata* leaves were collected in January 2015 from Kurungalam Village, Thanjavur district, Tamil Nadu from a single herb. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the Rapiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

### Preparation of extract:

The collected *Hydrophila auriculata* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The plant was dried at room temperature and coarsely powdered. The powder was extracted (Maceration) with methanol, ethanol and aqueous for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used. The extract contained both polar and non-polar phytocomponents of the plant material used.

### Phytochemical Screening

Preliminary phytochemical tests were carried out in the ethanolic extract of *Hydrophila auriculata* leaves using standardized procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

### Quantitative analysis of secondary metabolites by HPLC Analysis

**Sample preparation:** The extraction was carried out using 2 ml of fermented broth with 50 mL of 95% ethanol under 80 KHz, 45°C in ultrasonic extraction device for 30 min, repeated twice. The extract was collected and filtered; the filtrate was dried at 50°C under reduced pressure in a rotary evaporator. The dried crude extract was dissolved in 100 ml mobile phases. After filtering through a filter paper and 0.45 mm membrane filter (Millipore), the extract was injected into HPLC.

**HPLC conditions:** Samples were analysed using an RP-HPLC method [10], Shimadzu Corp., Kyoto, consisting of a LC-10ATVp pump, SCL 10A system controller and a variable Shimadzu SPD- 10ATVp UV VIS detector and a loop injector with a loop size of 20 µl. The peak area was calculated by a CLASSVP software. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250×4.6 mm i.d., particle size 5 µm, Luna 5µ C-18; phenomenex, Torrance, CA, USA) at 25°C. The gradient elution of solvent A [water-acetic acid (25:1 v/v)] and solvent B (methanol) had a significant effect on the resolution of compounds. As a result, solvent gradients were formed, using dual pumping system, by varying the proportion of solvent A [water-acetic acid

(25:1, v/v)] to solvent B (methanol). Solvent B was increased to 50% in 4 minutes and subsequently increased to 80% in 10 minutes at a flow rate of 1.0 mL/min. Detection wavelength was 280 nm.

**UV visible and FTIR Spectroscopic analysis**

The extracts were examined under visible and UV light for proximate analysis. For UV and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 minutes and filtered through Whatmann No. 1 filter paper by using a high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 260-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks ranging from 400-4000 $cm^{-1}$ and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation (Karpagasundari and Kulothungan ,2014).

**Results and Discussion**

The various bioactive phytochemical constituents available in plants include alkaloids saponins glycosides, flavonoids, phenol, coumarins, terpenes and carboxylic acids etc. These phytochemicals provide unique biological properties to plants. So, phytochemical analysis of the various constituents will be instrumental in determining the active agents responsible for biological activity.

In this present study the *Hydrophila auriculata* leaves extract was subjected to phytochemical screening and it is represented in table.1. Tannin, Saponnin, Flavonoids, Terpenoids, Triterpenoids, Carbohydrate, Polyphenol were present while Phlobatannins, Steroids, Alkaloids, Protein, Anthroquinone and Glycoside were absent in aqueous extract of *Hydrophila auriculata* leaves. Tannin, Saponnin, Flavonoids, Steroids, Alkaloids, Terpenoids, Triterpenoids, Anthroquinone, Carbohydrated and Polyphenol were present while Phlobatannins, Protein, and Glycoside were absent in methanol extract of *Hydrophila auriculata* leaves. Tannin, Saponnin, Flavonoids, Steroids, Alkaloids, Triterpenoids and Polyphenol were present while Phlobatannins, Terpenoids, Carbohydrate, Protein, Anthroquinone and Glycoside were absent in ethanol extract of *Hydrophila auriculata* leaves.

Significant quantity of phenol (204.23±14.28), Flavonoids (125.78±8.75), Alkaloids (68.77±4.80), Tannin (40.32±2.8) and Terpenoids (58.63±4.06) present in *Hydrophila auriculata* leaves and given in table 2. The leaves are rich in the phenol and flavonoid content when compared to alkaloids, tannins and terpenoids.

Spectroscopic (UV-Vis & FTIR) methods are very rapid and cost effective then other conventional methods, so both can be used together or separate in this manner to detect the bioactive constituents (Ibrahim et al., 2008). UV spectrophotometric analysis is a simple, rapid and accurate method for the determination of bioactive compounds present in crude drug powdered medicinal plants. The UV-Visible spectra were performed to identify the compounds containing σ- bonds, π-bonds, and lone pair of electrons, chromophores and aromatic rings The profile showed the peaks at 219.3, 230.5, 330.7, 412.5 and 659.5nm with the absorption 3.660, 1.257, 0.771,, 0.586 and 0.223 respectively (Table.3 & Fig.1). The result confirms the occurrence of peaks at 208-650nm reveals that the absorption bands (Liu *et al*., 2006) are due to the presence of flavonoids , phenol and its derivatives in the *Hydrophila auriculata* leaves**.**

The FTIR spectral analysis shows the presence of characteristic functional groups present in the leaves based on the peak value in the region of infra red radiation. When the leaves extract was passed in to the FTIR, the functional group of the components were separated based on its ratio. The peak values and the functional groups were represented in Table 4 & Fig.2. The results of the FTIR confirmed the presence of alcohol, phenol, Alkenes, Aromatics, Carboxylic acids, Aliphatic amines and Nitro compounds.

HPLC profiles of *Hydrophila auriculata* were analysed and seven phenolic compounds Vi2 Kaempferol, P-coumaric Acid, Epicatechin,Tannic acid, Epigallocatechin, Ellagic acid and Naringenin having different elution times could be obtained (Figure 2 and Table 2) when each compound was analyzed individually using the mobile gradient phase consisting of methanol and 1% acetic acid in water during 30 minutes run time.

The leaves of *Hydrophila auriculata* appear to be rich in secondary metabolites, widely used in traditional medicine to combact and cure the various ailments. Flavonoids are found to be abundant in the leaves and they protect against allergies, Platelet aggregation, microbial infections, Ulcers, hepatotoxins and tumors (Okwu and Omodamiro ,2005).

Flavonoids reduce the risk of estrogen induced cancers by interfering with the enzymes that produce estrogen(Donatus *et al*., 2009).They act as powerful protective agent against gastrointestinal infections, inflammations,Odema and inhibit the synthesis of Prostaglandin E2,F2 and thromboxane B2.They are powerful antioxidants and free radical scavengers, which prevent oxidative cell damage, and have strong anticancer activity They also inhibit microbes which are resistant to antibiotics. Presence of Saponins are responsible for the, foaming activity and cell membrane permeabilizing activity. Especially Saponins have haemolytic property , induce cytotoxic effect ,expectorant action ,antitumor,antimutagenic activities and can reduce the risk of cancers by preventing the cancer cells from growing. It also used to stop bleeding and in treating wounds(Okwu and Joshi ,2006). Traditionaly saponins are used as detergents, foaming ,surface active agents, pesticides,molluscides and have relationship with oxytocin which controls the onset of labor of women and the subsequent release of milk (Sri *et al.,*2004). Saponin have the ability to modulate the cell mediated immune system as well as enhance antibody production(Oda *et al.,* 2000).

Terpenoids posses membrane distruption and inhibitory effect against fungi and bacteria and also have inflammatory ,analgesic, anticancer, antimicrobial, antiviral, anti ulcer, hepaticidal and antitumor activities(Mahato and Sen ,1997). Anthocyanins help the immune system and protect the body against influenza virus .Various studies have proved that coumarins have strong antioxidant effect due to its ability to show both antimutagenic as well as anticarcinogen effect. Many coumarin derivatives act as free radical scavengers (Kontogirogis and Hadjiipavlou-Litina,2003) Non toxicity and high activity of several Coumarins was observed in the inhibition of carcinogenesis produced by benzo[*a*]pyrene(Wall *et al.,* 1998) . Generally Coumarins are highly potent anti inflammatory agent and directed against cell-adhesion molecule and they play as effective anticoagulants by inhibiting the function of Vitamin K which is essential for prothrombin biosynthesis (Goodman and Gilman's ,2006). Alkaloids posses plasmolytic, anticholinergic, analgesic, stimulants, antimalarials and anesthetic activity and reduces the fever and headache (Pietta, 2000)**.** Phenols are highly effective anticoagulants,antioxidants,immune enhancers, hormone modulators, and they modify the prostaglandin pathways, protect platelets from clumping and inhibits the enzymes which stimulates the inflammation(Duke ,1992) .Saponins and Glycosides shows antimicrobial activity (Illango *et al.,* 2012) . Glycosides are cardioprotective and used to treat the cardiac arrhythmia and congestive heart failure Tannins are moderately present and posses haemostatic activity, potential biological antioxidant ,antidiarrheal, antihemorrhoidal, antiviral, antiparasitic, (Kolodziej and Kiderlen, 2005) antibacterial, antifungal, proton precipitating agent and effective metal ion chelator (Okonkwo, 2009) . Apart from this tannins posses astringency property i.e. faster the healing of wounds and inflamed mucous membrane. The potential antimutagenic and anticarcinogenic activity has been related to their antioxidative property, which is important in protecting cellular damage including lipid peroxidation .Moderate amount of polyphenols posses cellular support and form the integral part of the cell wall. They are antiapoptotic,anti aging, anticarcinogenic, antiinflammatory, antiatherosclerotic, cardiovascular protective and prevent from oxidative stress as well as inhibition of angiogenesis and cell proliferation (Han *et al*., 2007).

**Conclusion:**

The results obtained in present study reveals that *Hydrophila auriculata* leaves extract posses various bioactive constituents like Flavonoids, phenols, saponins, terpinoids, , tannins, polyphenols, glycosides and alkaloids. Therefore screening intimates, presence of many bioactive chemical constituents which act as antiinflammatory, anticancer, antimicrobial, antioxidants, antidiarrheal and antihemorrhoidal agents and further investigation needs to elute novel

active compounds from the medicinal plants which may create a new way to treat many incurable diseases.

**Table 1: Preliminary phytochemical Screening of *Hydrophila auriculata* leaves extract**

| S.No | Secondary Metabolites | Aqueous Extract | Methanol Extract | Ethanol Extract |
|---|---|---|---|---|
| 1. | Tannin | + | + | + |
| 2. | Phlobatannins | --- | --- | --- |
| 3. | Saponnin | + | + | + |
| 4. | Flavonoids | + | ++ | + |
| 5. | Steroids | --- | + | + |
| 6. | Terpenoids | + | ++ | + |
| 7. | Triterpenoids | + | + | --- |
| 8. | Alkaloids | --- | + | + |
| 9. | Carbohydrate | + | + | --- |
| 10. | Protein | --- | --- | --- |
| 11. | Anthroquinone | --- | + | --- |
| 12. | Polyphenol | + | +++ | + |
| 13. | Glycoside | --- | --- | --- |

**(+) Presence; (--) Absence ++ = Medium, +++ = High concentrations,**

**Table 2: Quantitative analysis of Phytochemicals of *Hydrophila auriculata* leaves extract**

| S.No | Name of the Compound (mg Kg-1) | Observations |
|---|---|---|
| 1 | Phenol | 204.23±14.28 |
| 2 | Flavonoids | 125.78±8.75 |
| 3 | Alkaloids | 68.77±4.809 |
| 4 | Tannin | 40.32±2.8 |
| 5. | Terpenoids | 58.63±4.06 |

Values are expressed as ±Mean SD for triplicates

**Table:3. UV-VIS Peak Values of Extract of *Hydrophila auriculata* leaves**

| S.No | Wave length (nm) | Absorption peak |
|---|---|---|
| 1 | 219.3 | 3.660 |
| 2 | 230.5 | 1.257 |
| 3 | 330.7 | 0.771 |
| 4 | 412.5 | 0.586 |
| 5 | 659.5 | 0.223 |

**Table 4. FTIR Peak Values of Extract of *Hydrophila auriculata* leaves**

| S.No. | PEAK | Bond | Functional group |
|---|---|---|---|
| 1. | 3390.44 | O-H stretch, H-bonded | Alcohols, Phenols |
| 2. | 2976.86 | O-H stretch | Carboxylic acids |
| 3. | 2929.04 | O-H stretch | Carboxylic acids |
| 4. | 2900.36 | O-H stretch | Carboxylic acids |
| 5. | 2542.23 | O-H stretch | Carboxylic acids |
| 6. | 2255.90 | C=N stretch | Nitriles |
| 7. | 2133.56 | -C=C- stretch | Alkynes |
| 8. | 1651.84 | -C=C- stretch | Alkeynes |
| 9. | 1452.57 | C-C stretch (in-ring) C-H bend | Aromatics Alkanes |
| 10. | 1408.89 | C-C stretch (in-ring) | Aromatics |
| 11. | 1330.24 | N-O symmetric stretch | Nitro compounds |
| 12. | 1274.28 | C-N stretch | Aromatic amines |
| 13. | 1087.11 | C-N stretch | Aliphatic amines |
| 14. | 1049.50 | C-N stretch | Aliphatic amines |
| 15. | 881.0 5 | =C-H bend | Alkenes |
| 16. | 803.06 | =C-H bend | Alkenes |
| 17. | 675.77 | =C-H bend | Alkenes |

**Fig 1. UV-Vis Spectral analysis of Hydrophila auriculata leaves extract**

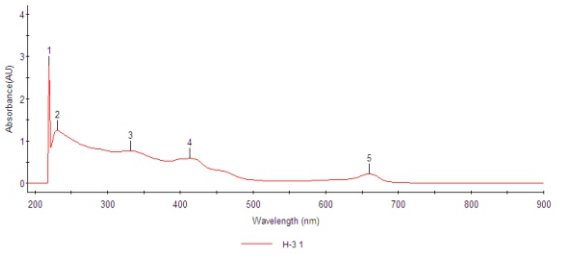
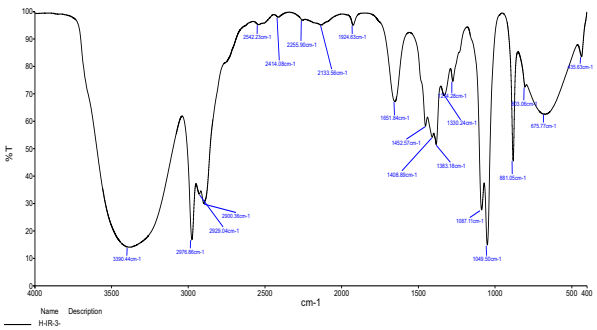



**Fig 2. FTIR analysis of Hydrophila auriculata leaves extract**



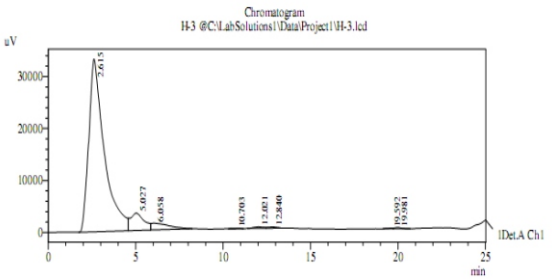




**Fig 3. HPLC chromatogram of Hydrophila auriculata leaves extract**

**Table 5 HPLC analysis of *Hydrophila auriculata* leaves extract**

| Peak# | Ret. Time | Area | Height | Area % | Height % | Compounds |
|---|---|---|---|---|---|---|
| 1 | 2.615 | 2047482 | 33260 | 87.055 | 85.524 | Kaempferol |
| 2 | 5.027 | 180367 | 3333 | 7.669 | 8.571 | Tannic acid |
| 3 | 6.058 | 90201 | 1293 | 3.835 | 3.325 | Epigallocatechin |
| 4 | 10.703 | 3026 | 94 | 0.129 | 0.242 | P-coumaric Acid |
| 5 | 12.021 | 12500 | 311 | 0.531 | 0.800 | Ellagic acid |
| 6 | 12.840 | 9106 | 240 | 0.387 | 0.617 | Epicatechin |
| 7 | 19.592 | 2793 | 119 | 0.119 | 0.307 | Salicylic acid |
| 8 | 19.981 | 6466 | 238 | 0.275 | 0.613 | Naringenin |
| Total | | 2351941 | 38890 | 100.000 | 100.000 | |

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