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Phytochemical Analysis of some Macrophytes of Lake Kondakarla, Visakhapatnam district, Andhra Pradesh

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Abstract: *Phytochemicals are secondary metabolites produced by all plants which has medicinal uses. The phytochemical analysis of leaf extracts in aqueous, ether and chloroform extracts of indigenous medicinally important plants of Marselia quadrifolia, Trapa natans, Ipomoea aquatica,eichornia crassipes, Pistia stratiotes, Nymphaea nouchalli, Aponogeton natans, Nelumbo nucifera, Hydrilla verticillata and Typha angustifolia were investigated. Quantitative phytochemical analysis was done for the presence of bioactive constituents such as phenols, saponins and flavonoids using standard methods. The results revealed that the selected plant species have some important bioactive components. Most of the fractions showed the presence of saponins. Total saponin content for the studied plant species ranged between 0.05±0.01 to 0.95±0.04. The highest saponin content was observed for the ethanol extract of Nelumbo nucifera (0.95±0.04) followed by Aponogeton natans (0.35±0.05) ethanol extract.*

Key Words: *Macrophytes, phytochemical, Phenols, Saponins, Flavonoids,Secondary metabolites.*

I. Introduction

The plants which have been selected for medicinal uses over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs (Dewick, 1996; Phillipson and Wright, 1996). According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga et al.,2005). The amount of phytochemical substances varies considerably from species to species and even from plant to plant, depending on the age and various ecological and climatic factors (Baquar, 1989) . Plants have limitless ability to synthesize aromatic substances, mostly phenols or their oxygen-substituted derivatives (Geisssman, 1963). Most of the natural products are secondary metabolites and about 12,000 of such products have been isolated so far. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro (Cowan, 1999). Plant products from barks, leaves, flowers, roots, fruits, seeds have been part of phytomedicines since time immemorial (Criagg and David, 2001). Knowledge of the chemical constituents of plants is desirable for synthesis of complex chemical substances (Mojab et al.,2003; Parekh and Chanda, 2007,2008). Today there is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacological activity. In the present work, qualitative and quantitative phytochemical analysis were carried out on some important Macrophytes.

II. Materials and Methods

Collection of plant materials

On the basis of the ethno-medicinal and rich pharmaceucital value ten macrophyte species were collected for biochemical analysis such as *Marselia quadrifolia, Trapa natans, Ipomoea aquatica,eichornia crassipes, Pistia stratiotes, Nymphaea nouchalli, Aponogeton natans, Nelumbo nucifera, Hydrilla verticillata* and *Typha angustifolia* from kondakarla lake.The collected plants were identified using available published literature (Ambasta ,1992; Chopra et al., 1956 & 1969).The plant materials were shade dried until all the water molecules evaporated and plants became well dried for grinding. After drying, the plant materials were ground well using mechanical blender into fine powder and transferred into airtight containers with proper labeling for future use.

***Study area*:**

The Kondakarla lake located north-east of Kondakarla village, lies between latitudes 17°35'30" and 17°36'02" N, and longitudes 82°01'0" E in Visakhapatnam District. The water spread area of the lake is about 6.5 km. It roughly measures Ca. 3 km in North-South direction and about 2.5 kms in the East-West direction. The lake has a relatively small catchment area, Ca 20 km. It is also mostly fed by hill stream and supply channel from river Sarada.

Preparation of plant extracts

Crude plant extract was prepared by Soxhlet extraction method. About 20gm of powdered plant material was uniformly packed into a thimble and extracted with 250ml of different solvents separately. Solvents used were methanol, ethanol, and acetone. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºC till all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC for their future use in phytochemical analysisof saponins, flavonoids and phenols.

III. Quantitative phytochemical analysis

Determination of total phenolic content

The amount of phenol in the aqueous extract was determined by Folin-Ciocalteu reagent method with some modifications. 2.5ml of 10% Folin-Ciocalteu reagent and 2ml of 2% solution of Na2CO3 were added to 1ml of plant extract. The resulting mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 765nm. Gallic acid was used as standard (1mg/ml). The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound) (Yadav and Munin,2011 ; Aiyegroro, 2010).

Determination of total flavonoid content

Aluminium chloride colorimetric method was used with some modifications to determine flavonoid content. 1ml of sample plant extract was mixed with 3ml of methanol, 0.2ml of 10% aluminium chloride, 0.2ml of 1M potassium acetate and 5.6ml of distilled water and remains at room temperature for 30 minutes. The absorbance was measured at 420nm. Quercetin was used as standard (1mg/ml). Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/g of extracted compound) (Yadav and Munin,2011 ; Aiyegroro,2010)

Determination of total saponin content

Total saponin determination was done using anisaldehyde reagent. Sample solution was prepared in water. For total saponins estimation 500 µl of sample, 500 µl of 0.5% anisealdehyde reagent, were mixed and kept aside for 10 min. Later, 2 ml of 50% sulphuric acid reagent was added and tubes were mixed. Tubes were then kept in water bath with constant temperature of 60°. After 10 min tubes were cooled and absorbance was taken at 435 nm. The amount of saponins was calculated as saponin equivalent from the calibration curve of standard saponin (100-1000 µg/ml) (Ing-Luen et al., 2009).

IV. Results And Discussion

The chloroform and ethanol extracts were analyzed for quantification of Total Phenol, total saponin and total flavonoid contents.

Total Phenol content

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh et al.,2007). They posses biological properties such as antiapoptosis, antiaging, anticarcinogen, anti inflammation, anti atherosclerosos, cardiovascular protection, improvement of endothelial function, inhibition of angiogenesis and cell proliferation activities (Han et al., 2007 ; Yadav and Munin,2011). There was a report on the presence of an antioxidant compound from aquatic plant I.aquatica (Nagendra et al.,2008 and Ali et al.,2008). Furthur presence of polyphenols in some indian vegetables was also reported(Daniel, 1989).

In the present study highest phenolic content was observed for the ethanol extract of *Nymphaea nouchalli* (840±0.04) and 645.3±0.04 for chloroform extract. *Aponogeton natans* also expressed considerable high phenolic content of 643.5±0.09 for the ethanol extract. The least phenolic content was observed for the ethanol and chloroform extracts of *Trapa natans* (58.5±0.01 for chloroform extract and 64.5±0.03 mg GAE/ml for the ethanol extract. The total phenolic content for all the studies ten macrophyte species ranged between 58.5±0.01 to 840±0.04 mg GAE/ml. the TPC content *of Nelumbo nucifera* chloroform extract is 315.8±0.08 and 580.6±0.04 for the ethanol extract. *Marselia quadrifolia, eichornia crassipes, pistia statiotes, Hydrilla verticillata* and *Typha angustifolia* resulted moderate phenolic content for both the chloroform and ethanol extracts. The extractability percentage was highest for ethanolic extract of *Ipomoea aquatica* (615±0.01). The total phenol content of the studied plants was presented in table.1 and figure.1. These results were supported by the reports in *I.aquatica* (Igwenyi et al.,2011) and in *C.asiatica* (Thangavel et al.,2011). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Krings and Berger, 2001).

Total Flavonoid content

Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Yadav and Munin, 2011;Marjorie, 1996). They also are effective antioxidant and show strong anticancer activities (Okwu, 2004; Del et al.,1997).

Flavonoids were also observed to be widely spread among all the studied macrophyte species except *Ipomoea aquatica*. Highest flavonoid content was present in ethanol extract of *Nelumbo nucifera* (58.6±0.05) and for the chloroform extract it was observed to be (47.5±0.05 QA mg/ml). it is followed by *Marselia quadrifolia* (52.6±0.04 for ethanol extract and 41.3±0.02 mg QE/ml for the chloroform extract and *Aponogeton natans* 52.6±0.06 for ethanol extract and 47±0.05 for the chloroform extract. The other species were observed to have moderate content.(table.3 &figure.3). Suman et al., studied the flavonoids in leaf powder extracts of *Holoptelea integrifolia* and *Celestrus emarginata* .

Total saponin content

The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation (Yadav and Munin, 2011). Saponins has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo et al.,2000; Okwu,2004). The total saponin content was measured by using the standard method of Van-Burden (1981) with modifications. Saponins were observed to be present in almost all the studied macrophytes. Most of the fractions showed the presence of saponins. Total saponin content for the studied plant species ranged between 0.05±0.01 to 0.95±0.04. The highest saponin content was observed for the ethanol extract of *Nelumbo nucifera* (0.95±0.04) followed by *Aponogeton natans* (0.35±0.05) ethanol extract. *Pistia stratoites, Nymphaea nouchalli*, *Eichorrnia crassipes* and *Typha angustifolia* have also exhibited considerable quantity concentration of saponin content for the ethanol extract. The least saponin content was observed for *Marselia quadrifolia* (Fig.2). The absence of saponin content in *C.asiatica* was also reported (Thangavel et al., 2011), whereas its presence was positive in our study. Similar results were reported in *R.dumatorum* (Neelu Singh, 2011).

The results revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the plants studied in the treatment of different ailments. Therefore, extracts from these plants could be seen as a good source for useful drugs. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants.

Table 1.– Total phenol content in GAE mg/ml concentration.

S.No.	Plant species	Total Phenol Content (mg/ml)	
		Chloroform Extract	Ethanol Extract
1	*Marselia Quadrifolia*	168.5±0.04	189±0.02
2	*Trapa natans*	58.5±0.01	64.5±0.03
3	*Ipomoea aquatica*	120.8±0.05	615±0.01
4	*Eichornia crassipes*	112.3±0.02	145.2±0.06
5	*Pistia stratiotes*	143.5±0.04	450.2±0.05
6	*Nymphaea nouchalli*	645.3±0.04	840±0.04
7	*Aponogeton natans*	231.4±0.03	643.5±0.09
8	*Nelumbo nucifera*	315.8±0.08	580.6±0.04
9	*Hydrilla verticillata*	114.2±0.04	126.2±0.02
10	*Typha angustifolia*	148.5±0.01	275.6±0.01

Table.2.– Total Saponin content in mg/ml concentration

S.No.	Plant species	Total Saponin content (mg/ml)	
		Chloroform Extract	Ethanol Extract
1	*Marselia Quadrifolia*	0.05±0.01	0.12±0.03
2	*Trapa natans*	0.15±0.08	0.22±0.01
3	*Ipomoea aquatica*	0.12±0.02	0.19±0.05
4	*Eichornia crassipes*	0.14±0.03	0.25±0.02
5	*Pistia stratiotes*	0.16±0.01	0.2±0.01
6	*Nymphaea nouchalli*	0.16±0.01	0.24±0.04
7	*Aponogeton natans*	0.22±0.04	0.35±0.05
8	*Nelumbo nucifera*	0.5±0.05	0.95±0.04
9	*Hydrilla verticillata*	0.13±0.04	0.18±0.05
10	*Typha angustifolia*	0.14±0.02	0.22±0.03

Table.3.– Total Flavonoid content in mg/ml concentration

S.No.	Plant species	Total Flavonoid content (mg/ml)	
		Chloroform Extract	Ethanol Extract
1	*Marselia Quadrifolia*	41.3±0.02	52.6±0.04
2	*Trapa natans*	38.4±0.04	6.7±0.05
3	*Ipomoea aquatica*	--	--
4	*Eichornia crassipes*	31.2±0.05	39±0.04
5	*Pistia stratiotes*	12.3±0.06	19.5±0.02
6	*Nymphaea nouchalli*	35.6±0.09	46.8±0.03
7	*Aponogeton natans*	47±0.05	52.6±0.06
8	*Nelumbo nucifera*	47.5±0.07	58.6±0.05
9	*Hydrilla verticillata*	32.1±0.05	38.4±0.04
10	*Typha angustifolia*	22.5±0.01	34.9±0.07

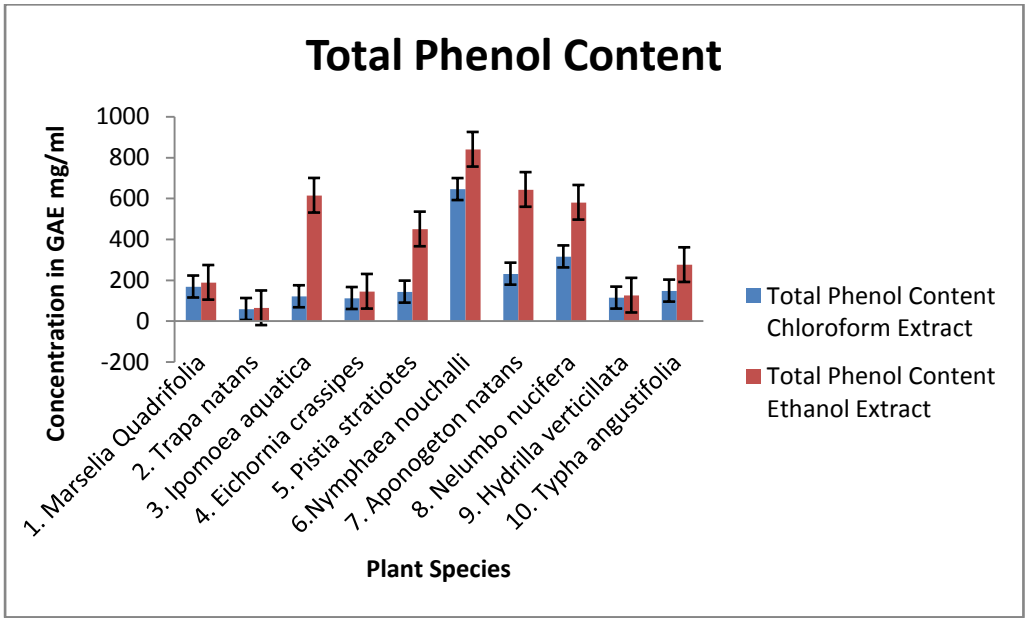

Fig.1. Quantification of Total Phenol Content

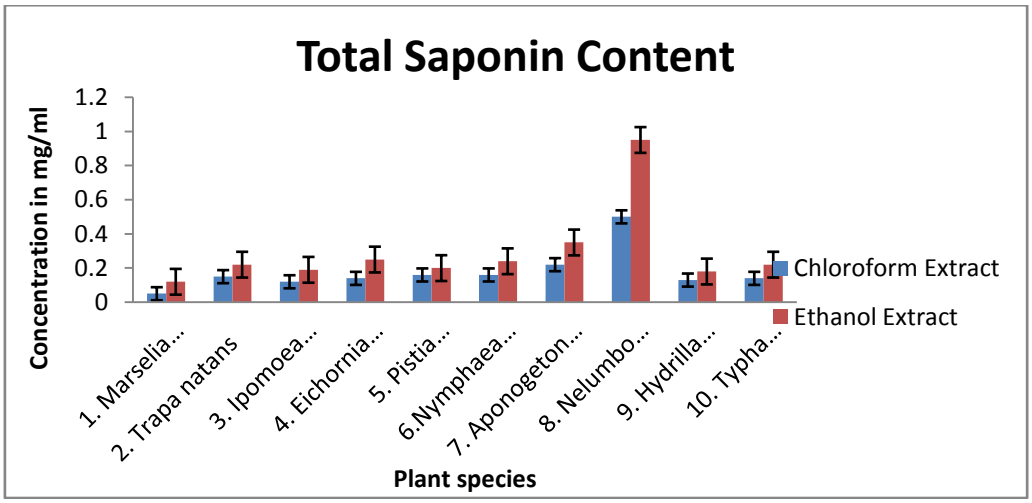

Fig.2. Quantification of Total Saponin Content

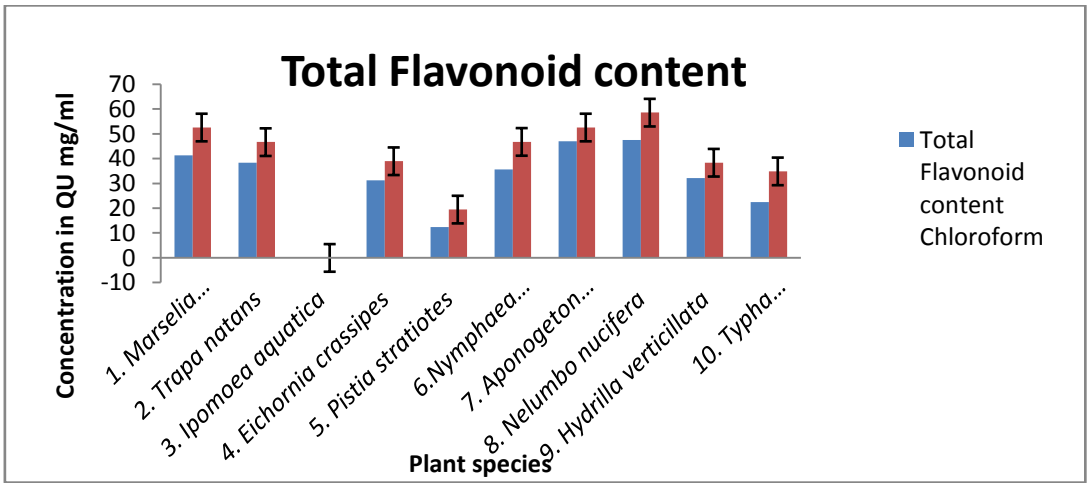

Fig.3. Quantification of Total Flavonoid Content

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