ISOLATION AND IDENTIFICATION OF FLAVONOIDS FROM AERIAL PARTS OF *HELIOTROPIUM INDICUM*.

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Abstract

Heliotropium indicum is an aromatic plant which is well known for its characteristic fragrance anddistinct aroma. In the present study flavonoids have been extracted from dried and powdered samples of stem,leaves, and flowers of *Heliotropium indicum* by well established method. Free (ether fraction) and bound (ethyl acetate) fraction of flavonoids were extracted from different parts of plants and were separately dried andweighed. Results reveal that free flavonoids were maximum in flowers (0.006m/g.dw) and bound flavonoids were maximum in leaves (0.007m/g.dw). However total flavonoids (free+ bound) content was maximum in leaves (0.008m/g.dw) followed by flowers (0.007m/g.dw) and stem (0.002m/g.dw).

Keywords: *Heliotropium indicum*, Flavonoids.

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Introduction

Heliotropium indicum is an aromatic plant which is well known for its characteristic fragrance and distinct aroma. In the present study flavonoidshave been extracted from dried and powderedsamples of stem, leaves, and flowers of *Heliotropium indicum* by well established method. Free (ether fraction) and bound (ethyl acetate) fraction of flavonoids were extracted from different parts of plants and were separately dried and weighed. Results reveal that free flavonoids content was maximum in flowers (0.006m/g.dw) and bound flavonoids were maximum in leaves (0.007m/g.dw). However total flavonoids (free+ bound) content was maximum in leaves (0.008m/g.dw) followed by flowers (0.007m/g.dw) and stem (0.002m/g.dw)[1].

Materials and Methods

Plant material *Heliotropium indicum* was collected from State Institute of Rural Development (SIRD), Kahikuchi, Guwahati. A voucher specimen (017823) has beendeposited in the Herbarium, department of Botany, Gauhati University[2, 3].

Flavonoid extraction

Plants collected were washed in running tapwater to remove dust. Aerial part (stems, leaves,flower) of collected plants were separated, shadedried powdered weighed and stored separately forextraction. Each of the dried powdered and weighed sample was soxhlet extracted in 80% methanol for24 hrs and filtered. The filtrate obtained from eachsample was subsequently extracted in petroleum ether, diethyl ether and ethyl acetate following themethod of Subramanian and Nagarajan (1969). Petroleum ether fraction was discarded due to itsbeing rich in fatty substances. Ether fraction wasused for free flavonoids whereas ethyl acetate fraction for bound flavonoids. Ethyl acetate fractionof each sample was hydrolysed further with 7% H_2SO_4 for 24 hrs and was then re-extracted withethyl acetate. The fraction obtained was repeatedlywashed with distilled water to neutrality, dried andweighed[4].

Qualitative Thin layer chromatography

Thin glass plates (20x20cm) were coatedwith Silica gel G (250 mμ thick) and were dried atroom temperature. Thereafter were kept at 100°Cfor 30 minutes for activation and were then cooledat room temperature prior to loading of sample. Eachof the extract was co-chromatographed withauthentic flavonoid samples of quericetin and kaempferol. The plates were developed in an airtightchromatographic chamber saturated withsolvent mixture, Benzene: Acetic Acid: Water (125:72:3). Developed plates were air dried andvisualized under UV light and were also exposed toiodine vapors for preliminary detection. Plates were also exposed to NH_4OH bottle so as to make contactwith each spot for about 5-10 seconds andfluorescent spots corresponding to that of standardflavonoids were marked. The developed plates werealso sprayed with 5% FeCl3, 0.1% alcoholic AlCl3for further confirmation. The coloured spots thus developed were noted and the Rf values of each spotwas calculated. Several other solvent systems such as n-butanol, acetic acid, water (4:1:5, upper layer), n-butanol, acetic acid, water (3:1:1) were also

tried, but the solvent system containing benzene, aceticacid and water (125:72:3) gave best results. Hencewas used for PTLC (Preparative Thin Layer Chromatography)[5].

Preparative Thin Layer Chromatography (PTLC)

PTLC was performed with about 250 silicagel G coated plates (0.4 - 0.5mμ) These plates were developed in benzene:aceticacid:water (125:72:3)air-dried and examined under UV light. Each spot of Rf values of standards were marked and eluted. The eluted compounds were subjected to crystallization separately and their melting point, mixed melting point were determined. The isolated compounds were also subjected to IR spectral studies along with standard reference compounds for confirmation[6].

Identification

Melting point of kaempferol(276-278),Mixed melting point and IR spectra of theisolated compound was taken and also on the basisof preliminary detection and confirmation withstandard compounds were identified as quercetin and kaempferol.

Results and Discussion

Flavonoids have been extracted from stem, leaves and flowers of the selected plant (*Heliotropium indicum*). Free (ether fraction) and bound (ethylacetate fraction) flavonoids of different plant parts (stem, flowers, leaves) were extracted dried andweighed (Table1). Results reveal that maximum freeflavonoids were obtained from flowers (0.006mg/gdw) and maximum bound flavonoidswere obtained from leaves (0.007mg/gm.drywt).However total flavonoids (free+bound) were foundto be maximum in leaves (0.008mg/gdw), followed by flowers (0.007mg/gdw) and stem (0.002mg/gdw) (Figure 1). On the basis of Rf values Kaempferol (0.95) has been identified in thedifferent parts of plant. HPLC analysis furtherconfirms the presence of Kaempferolin the plant[7,8]. Kaemoferol was identified by the IR spectroscopy (figure 2).

Conclusion

In the above study we can conclude that byquantitative analysis of flavonoid of aerial parts of *Heliotropium indicum*. Maximum freeflavonoids were obtained from flowers (0.006mg/gdw) and maximum bound flavonoidswere obtained from leaves (0.007mg/gm.drywt).However total flavonoids (free+bound) were foundto be maximum in leaves (0.008mg/gdw), followed by flowers (0.007mg/gdw) and stem (0.002mg/gdw) Table1. On the basis of Rf values Kaempferol(0.95) and Quercetin (0.78) have been identified inthe different parts of plant. HPLC analysis further confirms the presence of Kaempferol and Quercetin in the plant.

Table: 1 : **Flavonoid content from aerial Parts of *Heliotropium indicum***

Sl.No	Parts	Free Flavonoids mg/g DW	Bound Flavonoids mg/g DW	Total Flavonoids mg/g DW
1	Stem	0.001	0.001	0.002
2	Leaves	0.001	0.007	0.008
3	Flowers	0.006	0.001	0.007

Flavonoids content of *Heliotropium indicum*

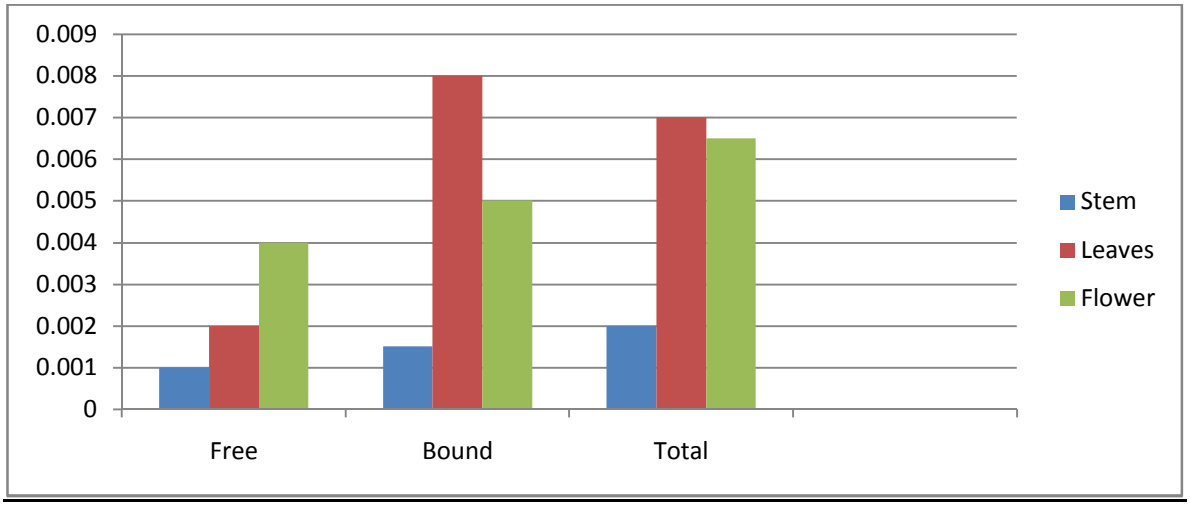

Figure 1. : Graphical representation of Flavonoid Content

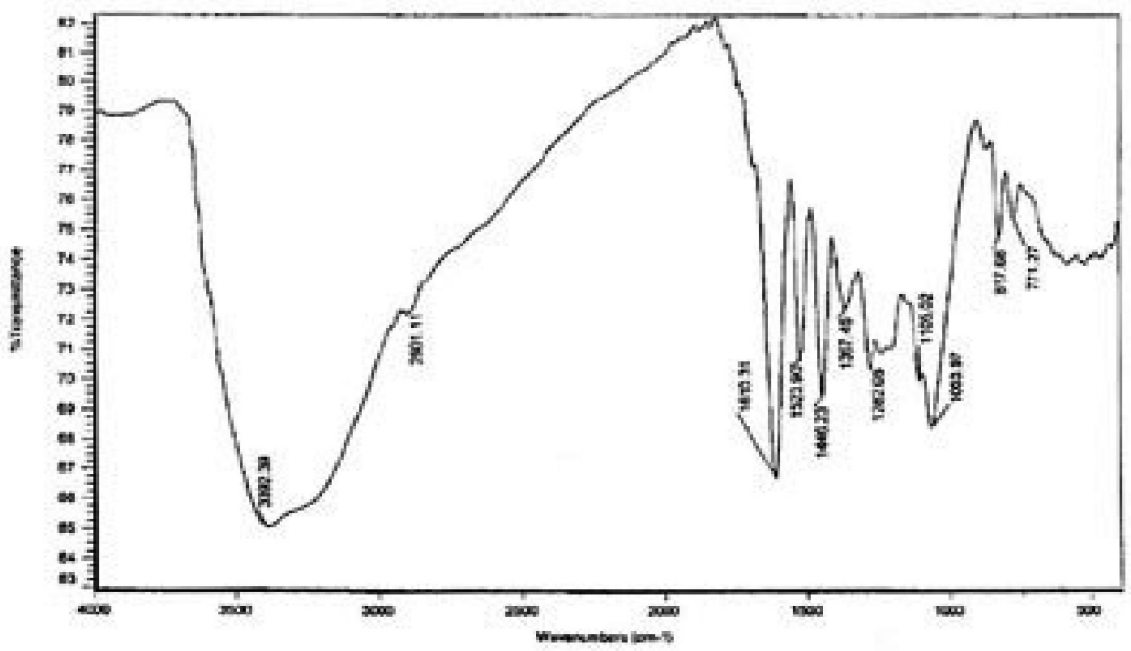

Fig 2.: IR for the Isolated Kaempferol

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Authors' contributions

Mr. Debaprotim Dasguptahas carried out the entire work starting from collection of plant material; it's processing for extraction, phytochemical screening, determination of anticancer activity, interpretation of the results and drafting the manuscript.

Mr. Saumendu Deb Roy has contributed in dosing the animal for anticancer study and has given valuable suggestions and instructions for performing the research work. The final review of the manuscript was done by him.

All authors read and approved the final manuscript.

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