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PRODUCTION, ISOLATION AND IDENTIFICATION OF FLAVONOIDS FROM AERIAL PARTS OF *HIPTAGE BENGHALENSIS*

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ABSTRACT

Hiptage benghalensis (L) Kurz is an aromatic plant which is well known for its characteristic fragrance and distinct aroma. In the present study flavonoids have been extracted from dried and powdered samples of stem, leaves, and flowers of *Hiptage benghalensis* 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 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: *Hiptage benghalensis*, flavonoids.

INTRODUCTION

Hiptage benghalensis (L) Kurz is an aromatic plant which is well known for its characteristic fragrance and distinct aroma. In the present study flavonoids have been extracted from dried and powdered samples of stem, leaves, and flowers of *Hiptage benghalensis* 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 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).

MATERIALS AND METHODS

Plant material

Hiptage benghalensis was grown and maintained in the Department of Botany, University of Rajasthan, Jaipur following standard agronomic practices.A voucher specimen **(RUBL No - 20863)** has been deposited in the Herbarium, department of Botany, University of Rajasthan.

Flavonoid extraction

Plants collected were washed in running tap water to remove dust. Aerial part (stems, leaves, flower) of collected plants were separated, shade dried powdered weighed and stored separately for extraction. Each of the dried powdered and weighed sample was soxhlet extracted in 80% methanol for 24 hrs and filtered. The filtrate obtained from each sample was subsequently extracted in petroleum ether, diethyl ether and ethyl acetate following the method of **Subramanian** and **Nagarajan** (1969). Petroleum ether fraction was discarded due to its being rich in fatty substances. Ether fraction was used for free flavonoids whereas ethyl acetate fraction for bound flavonoids. Ethyl acetate fraction of each sample was hydrolysed further with 7%

H_2So_4 for 24 hrs and was then re-extracted with ethyl acetate. The fraction obtained was repeatedly washed with distilled water to neutrality, dried and weighed.

Qualitative

Thin layer chromatography

Thin glass plates (20x20cm) were coated with Silica gel G (250 mμ thick) and were dried at room temperature. Thereafter were kept at 100°C for 30 minutes for activation and were then cooled at room temperature prior to loading of sample. Each of the extract was co-chromatographed with authentic flavonoid samples of quericetin and kaempferol. The plates were developed in an air-tight chromatographic chamber saturated with solvent mixture, Benzene: Acetic Acid: Water (125:72:3). Developed plates were air dried and visualized under UV light and were also exposed to iodine vapours for preliminary detection. Plates were also exposed to NH_4OH bottle so as to make contact with each spot for about 5-10 seconds and fluorescent spots corresponding to that of standard flavonoids were marked. The developed plates were also sprayed with 5% $FeCl_3$, 0.1% alcoholic $AlCl_3$ for further confirmation. The coloured spots thus developed were noted and the Rf values of each spot was 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, acetic acid and water (125:72:3) gave best results. Hence was used for PTLC (Preparative Thin Layer Chromatography).

Preparative Thin Layer Chromatography (PTLC)

PTLC was performed with about 250 silica gel G coated plates (0.4 - 0.5mμ) These plates were developed in benzene:acetic acid: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**.**

Identification

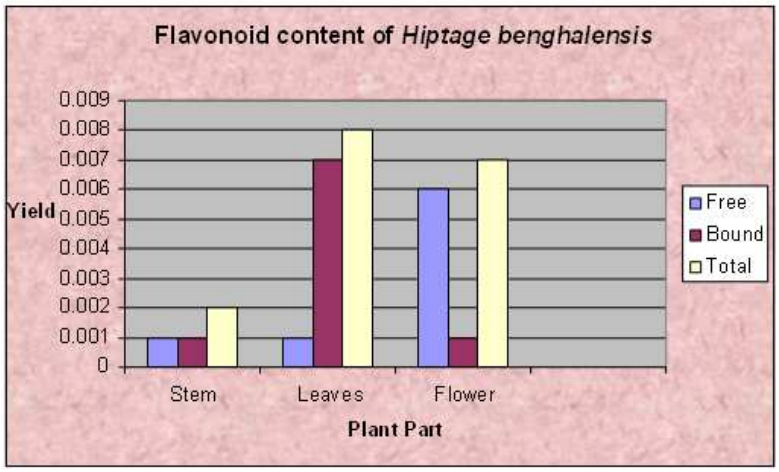
Melting point of kaempferol (276-278), Mixed melting point and IR spectra of each of the isolated compound was taken and also on the basis of preliminary detection and confirmation with standard compounds were identified as quercetin and kaempferol.

RESULTS AND DISCUSSION

Flavonoids have been extracted from stem, leaves and flowers of the selected plant (*Hiptage benghalensis*). Free (ether fraction) and bound (ethyl acetate fraction) flavonoids of different plant parts (stem, flowers, leaves) were extracted dried and weighed (Table1). Results reveal that maximum free flavonoids were obtained from flowers (0.006mg/gdw) and maximum bound flavonoids were obtained from leaves (0.007mg/gm.drywt). However total flavonoids (free+bound) were found to 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 in the different parts of plant. HPLC analysis further confirms the presence of Kaempferol and Quercetin in the plant.

Flavonoid content from aerial Parts of *Hiptage benghalensis*

S.NO	Parts	Free flavonoid m/g.dw	Bound flavonoids m/g.dw	Total flavonoids m/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



Graphical Representation of Flavonoid content of Hipage be nghalensis

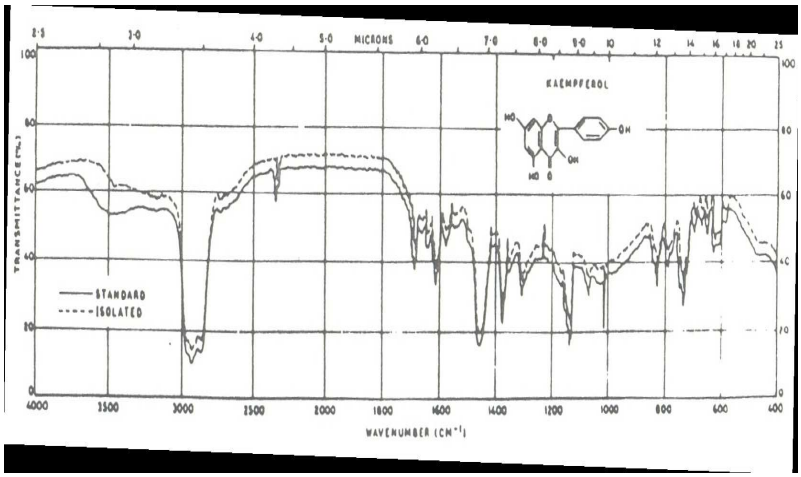


Figure 1.1 : ***IR : Infra Red Spectra of Isolated Kaempferol***

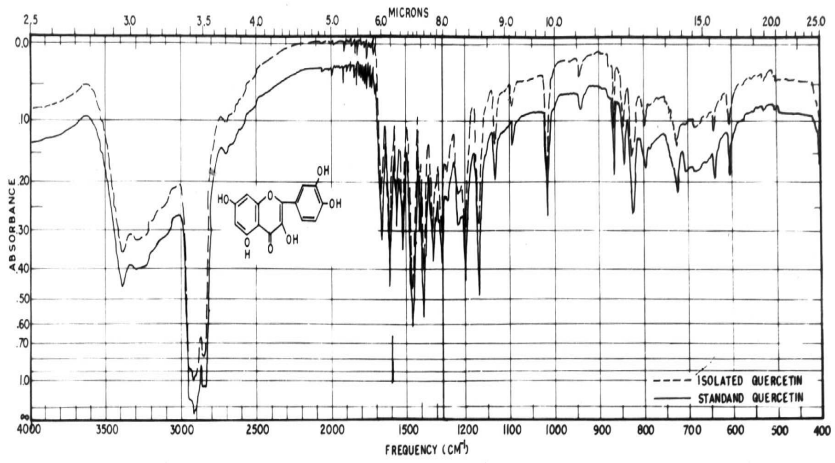


Figure 1.1 : ***IR : Infra Red Spectra of Isolated Quercetin***

CONCLUSIONS

In the above study we can conclude that by quantitative analysis of flavonoid of aerial parts of *Hiptage benghalensis*. That maximum free flavonoids were obtained from flowers (0.006mg/gdw) and maximum bound flavonoids were obtained from leaves (0.007mg/gm.drywt). However total flavonoids (free+bound) were found to 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 in the different parts of plant. HPLC analysis further confirms the presence of Kaempferol and Quercetin in the plant.

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