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Estimation of total phenolic and flavonoid content of *Hibiscus furcatus* Roxb leaves

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ABSTRACT

Hibiscus furcatus Roxb belonging to family, Malvaceae, is a herb, used in traditional medicine for the treatment of inflammation, hyperdyspsia, renal diseases etc. Ethanolic extract of *Hibiscus furcatus* was fractionated using different solvents of increasing polarity like petroleum ether, chloroform and ethylacetate. The preliminary phytochemical screening showed the presence of carbohydrates, steroids, flavonoids.

Keywords: *H.furcatus*, Total phenolic content, Total flavanoid content, Folin cio-calteau method, Gallic acid

INTRODUCTION

The use of traditional medicine is expanding to newer horizons and plants still remains as the novel source of structurally important compounds that leads to the development of innovative drugs. The main advantage of herbal drugs are low/minimum cost, potency and efficiency, enhanced tolerance, more protection, fewer side-effects, complete accessibility [1].

Hibiscus furcatus is an erect or trailing prickly herb. 2-5 ft height, growing throughout India. The plant flowers in September to November. The flowers are large and yellow with purple centre. The leaves of *Hibiscus furcatus* are acidic and are edible after cooking. The leaves are said to improve digestion and are considered as antihelmintic [2]. The juice of the leaves mixed with honey is applied in eye diseases. An infusion of the roots in water is

used as a refreshing drink in hot weather. A decoction of the root bark is given as remedy for poisons and swellings and for cleansing kidneys. It is used by tribal healers of Kerala to treat liver diseases [3]. The aim of the present study is to estimate the total phenolic and flavanoid content of *Hibiscus furcatus* leaves.

MATERIALS AND METHODS

Plant materials--- Air dried herbs of *Hibiscus furcatus* were obtained from Moorkanad, Trissur district, Kerala. Leaves were identified and authenticated by Dr.Jomy Augustine, Professor, Department of Botany, St. Joseph College, Pala with a voucher specimen (2260 dated 22/03/2013)

PREPARATION OF VARIOUS EXTRACTS

Shade dried and powdered leaves (100g) of *Hibiscus furcatus* was soaked in rectified spirit in a one litre round bottom flask. After soaking it for one day, it was refluxed with ethanol 95% (500ml) for 3 hours and the clear solution was decanted off. The extraction was repeated thrice. The combined extract was concentrated to a semisolid consistency. Thus total ethanolic extract was obtained. The fractionation of the ethanolic extract was carried out using solvents in the increasing order of polarity, i.e. Petroleum ether, chloroform and ethyl acetate. Each fraction was concentrated, weighed and stored for further study.

ESTIMATION OF TOTAL PHENOLIC CONTENT

Method

Folin Cio-calteau method [4]

Materials required

- Gallic acid
- Folin Cio-calteau reagent
- 7.5% Sodium carbonate
- Distilled water
- UV-VISIBLE Spectrophotometer (Shimadzu Model: UV 1800)
- Sample

Preparation of reagents

Folin cio-calteau reagent (2N) – 1:10 ratio with distilled water

Sodium carbonate – 7.5 g in 100ml distilled water

Preparation of standard solution

10mg gallic acid was weighed and made up to 10ml with methanol in a 10ml standard flask. From the above solution (1mg/ml), 1ml was pipetted out and made upto 10ml with methanol to get 100µg/ml gallic acid standard solution (stock solution). From the stock solution, 0.25, 0.50, 0.75 and 1.0ml was pipetted out and made up to 2ml with water to get, 25, 50, 75, and 100 µg/ml solutions respectively. To the above solutions, 5ml of Folin-Ciocalteu reagent was added and 4ml of 7.5% sodium carbonate solution was added after 5 minutes. It was stirred and incubated at room temperature for 2 hours. A reagent blank was also prepared using 1

ml of distilled water instead of gallic acid. After 2 hours, absorbance of the solutions was measured at 765nm using Shimadzu UV-VIS spectrophotometer. The absorbance values were plotted against concentration and standard graph was obtained. The experiments were performed in triplicate.

Preparation of sample solution

20mg of the extracts were weighed, dissolved in methanol and made up to 50ml with methanol (400µg/ml). 1ml was pipetted out from each extract solution and 5ml of Folin-Ciocalteu reagent was added. After 5 minutes, 4ml of Sodium carbonate solution was added and incubated at room temperature for 2 hours.

Then, absorbance was measured at 765nm and the values obtained were interpreted in the standard graph of Gallic acid to get the milligram equivalents of Gallic acid. The experiments were performed in triplicate.

ESTIMATION OF TOTAL FLAVANOID CONTENT

Method- aluminum chloride colorimetric method [5]

Materials required

- Rutin / Quercetin
- Sodium nitrite (5%)
- Aluminium chloride (10%)
- Sodium hydroxide (1M)

Preparation of reagents

Sodium nitrite (5%) – 5g in 100ml distilled water

Aluminium chloride (10%) – 10g in 100ml distilled water

Sodium hydroxide (1M) – 4g in 100ml

Preparation of standard graph of rutin

50mg rutin was weighed and made up to 50 ml with methanol in a 50 ml standard flask. From this stock solution 0.1, 0.2, 0.3, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml was pipetted out to get 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml solutions respectively. To each of this solution, 4 ml water was added followed by 0.3 ml of 5%sodium nitrite. After 5 minutes 0.3 ml of 10% aluminium chloride was added and at the 6th minute 1 ml of 1M sodium hydroxide was added. Then total volume

was made up to 10 ml with distilled water. A blank was prepared without rutin. The solutions were mixed and the absorbance was measured against the blank at 510 nm. A standard graph was plotted using concentration and absorbance. The results were tabulated in table:

Preparation of sample solution

20 mg of sample extract was weighed, dissolved in methanol and made up to 50 ml with methanol. 1ml was pipetted out from each dissolved samples and 4 ml of water followed by 0.3 ml of sodium nitrite was added. After 5 minutes, 0.3 ml of 10% aluminium chloride was added and at the 6th minute 2 ml of 1M sodium hydroxide was added. Mixed well and the absorbance was measured at

510 nm and the values were interpreted in the standard graph of rutin to get the mg equivalent of rutin. [5].

RESULTS

Estimation of Total Phenolic Content

The standard graph was plotted using various concentrations of gallic acid. The total phenolic content in the extract was determined and expressed as Gallic acid equivalents (mg/g). The absorbance values obtained for different concentrations of standard was tabulated and the standard graph was plotted.

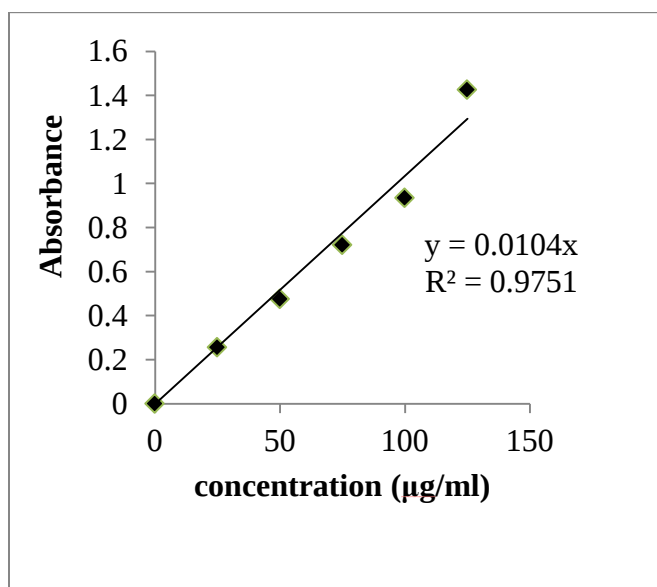
Absorbance values obtained for different concentrations of standard

Sl.No.	Concentration of Gallic acid($\mu\text{g/ml}$)	Mean Absorbance*
1.	25	0.256 $\pm$ 0.002
2.	50	0.476 $\pm$ 0.001
3.	75	0.721 $\pm$ 0.002
4.	100	0.934 $\pm$ 0.003
5	125	1.425 $\pm$ 0.0005

*Values are expressed as Mean $\pm$ SD, n=3

A calibration curve of gallic acid was plotted using Absorbance versus Concentration

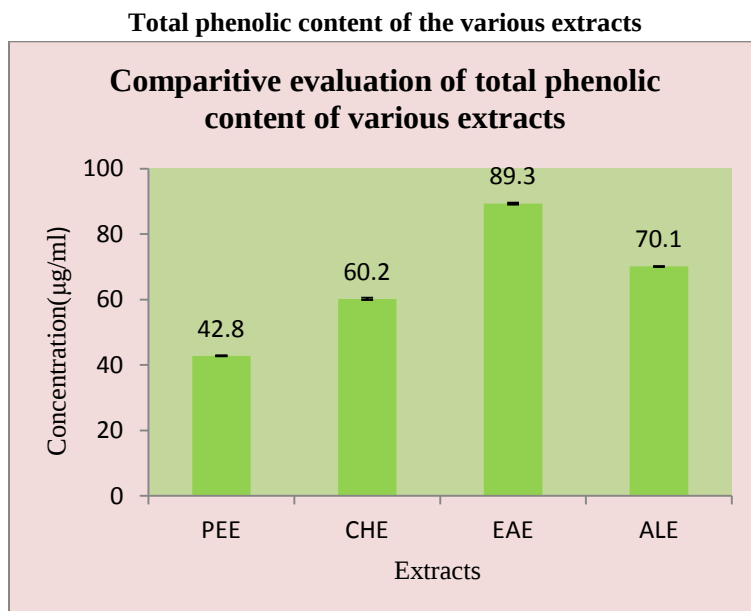
Standard Graph of Gallic acid



The absorbance values obtained for the extracts were recorded and the total phenolic content in the Total phenolic content of the various extracts

extracts were calculated as Gallic acid equivalent and tabulated in table given below.

Sl.No	Extracts	Mean Absorbance* (750nm)	Concentration from graph ($\mu\text{g/ml}$)*	Gram equivalent of gallic acid per 100g
1.	PEE	0.427 $\pm$ 0.003	42.8 $\pm$ 0.11	14.2
2.	CHE	0.595 $\pm$ 0.002	60.2 $\pm$ 0.31	19.8
3.	EAE	0.888 $\pm$ 0.003	89.3 $\pm$ 0.05	29.6
4.	ALE	0.69 $\pm$ 0.001	70.1 $\pm$ 0.30	23



From the graph it was observed that, ethyl acetate extract (EAE) have higher phenolic content when compared with other extracts.

Estimation of Total Flavonoid Content

The total flavonoid content in the extracts was determined by Aluminium chloride colorimetric

method. A standard graph of Rutin was plotted and the total flavonoid content was determined and expressed as Rutin equivalents (mg/g). The absorbance values obtained for different concentrations of standard was tabulated in following table.

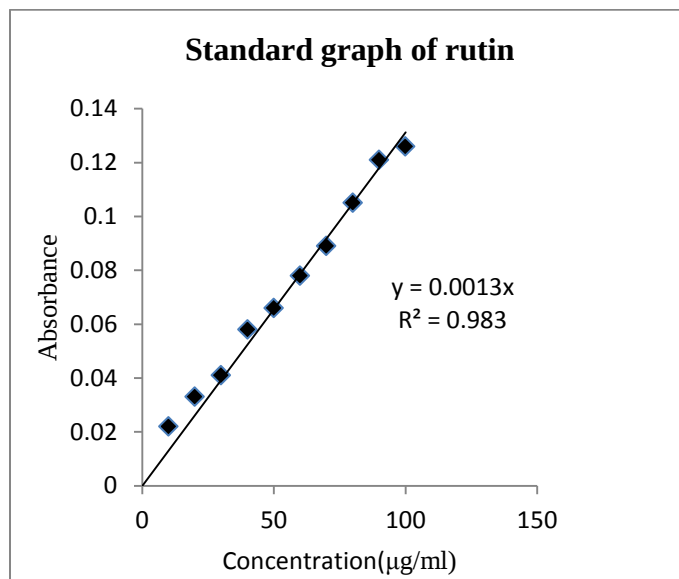
Absorbance values observed at different concentrations of standard

Sl.No	Concentration of Rutin ($\mu\text{g/ml}$)	Mean absorbance*
1.	10	0.023 $\pm$ 0.001
2.	20	0.036 $\pm$ 0.002
3.	30	0.040 $\pm$ 0.001
4.	40	0.058 $\pm$ 0.003
5.	50	0.066 $\pm$ 0.002
6.	60	0.078 $\pm$ 0.001
7.	70	0.088 $\pm$ 0.001
8.	80	0.105 $\pm$ 0.001
9.	90	0.121 $\pm$ 0.002
10.	100	0.126 $\pm$ 0.002

*Values are expressed as Mean $\pm$ SD, n=3

A calibration curve of rutin was plotted using concentration on the X-axis and absorbance on the Y axis.

Standard graph of Rutin



The absorbance values obtained for the extracts were recorded and the total flavonoid content in the

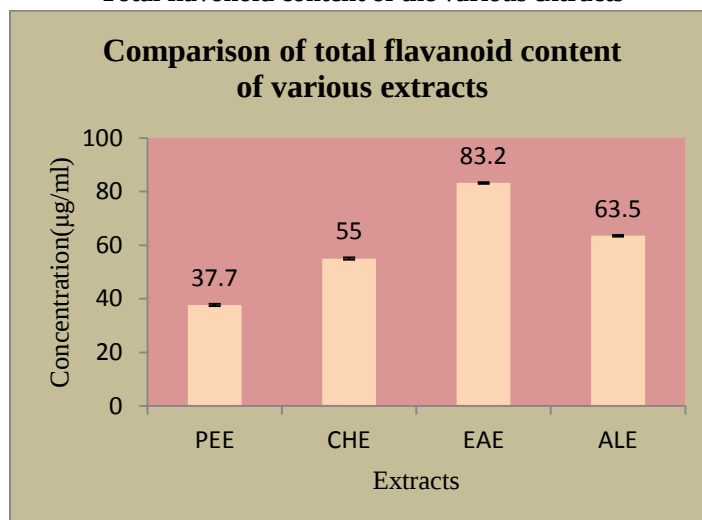
extracts were calculated, and tabulated in the following table

Total flavonoid content of the various extracts

Sl.No	Extracts	Absorbance* (510nm)	Concentration from the graph (µg/ml) *	Rutin equivalent per 100 gm
1.	PEE	0.047±0.001	37.7±0.46	3.7
2.	CHE	0.070±0.001	55±0.44	5.5
3.	EAE	0.115±0.002	83.2±0.30	8.3
4.	ALE	0.084±0.001	63.5±0.31	6.3

*Values are expressed as Mean±SD, n=3

Total flavonoid content of the various extracts

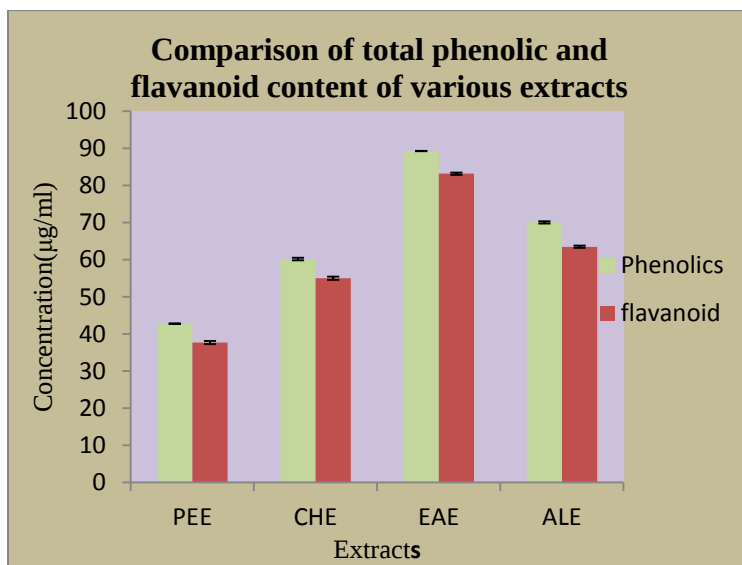


From the above graph it was observed that, ethyl acetate extract (EAE) have higher flavonoid content when compared with the other extracts.

The comparative representation of total phenolic content and flavonoid content of various

extracts, was depicted in the graph drawn below. From this, it was observed that, the ethyl acetate (EAE) extract have higher phenolic content and have higher flavonoid content when compared with other extracts.

Comparative evaluation of total phenolic and flavonoid content



DISCUSSION

Extraction is the most important step in phytochemical study and the extractive yield depends on solvent, time and temperature as well as the chemical nature of sample. Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive or inert components by using suitable solvents. In the present study extraction of leaves of *Hibiscus furcatus* with ethanol under reflux gave an yield of 22.70%w/w. Since ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material, is volatile and has a low toxicity to the bioassay it is a very useful extractant.

The total ethanolic extracts were fractionated with solvents of increasing polarity to obtain petroleum ether, chloroform, ethylacetate extract and alcoholic residue. The total ethanolic extract and four other extracts were subjected to preliminary phytochemical screening. The chemical

tests showed the presence of carbohydrate, phenolics, flavanoids, terpenoids and glycosides. Alkaloids were absent in the leaf extracts.

The estimation of total phenolic and flavonoids content indicated that *Hibiscus furcatus* leaves are rich in phenolics and flavonoids compounds. Ethyl acetate extract had shown high phenolic content compared to all the other extracts. Phenolics are found in large quantities in the plant kingdom, and they possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticancer as well as ability to modify the gene expression.

CONCLUSION

The different pharmacological actions of *Hibiscus furcatus* may be attributed to the presence of flavonoids, however further studies to characterize the active principles and to elucidate the mechanism is required.

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