QUALITATIVE AND QUANTITATIVE ANALYSIS AND DETERMINATION OF ANTIOXIDANT POTENTIAL OF FLAVONOIDS FROM LEAVES OF *HYPTIS SUAVEOLENS*

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ABSTRACT: *Flavonoids are water soluble polyphenolic molecules abundant in plants. They have strong antioxidant activity and are responsible for lowering cellular oxidative stress in human beings.Hyptis suaveolens (L.) is an obnoxious weed possessing many medicinal properties. In the current study, free and bound flavonoids were extracted from the leaves of Hyptis suaveolens, quantitatively estimated by Aluminium trichloride colorimetric method. Qualitative analysis was done by Thin layer chromatography (TLC) techniques using Quercetin as reference. 2, 2-Diphenyl-1-Picryl-Hydrazyl-Hydrate (DPPH) assay was carried out to evaluate the antioxidant potential of theextracted flavonoids. The bound flavonoids were found to be having higher concentration (20 mgQE/gm) thanthat of free Flavonoids. The Rfvalue of the bound flavonoids was found to be perfectly matching with that of Quercetin. Both the bound and the free flavonoids have excellent DPPH radical scavenging ability of 85% and 81% respectively.*

Key Words: *Flavonoids, Antioxidants, Hyptis suaveolens, radicalscavenging ability.*

INTRODUCTION

*Hyptis suaveolens*belonging to the family Lamiaceae is a very common plant found along roadsides and farms in different parts of the world . The plant is medicinally valuable. Almost all parts of this plant are being used in traditional medicine to treat various diseases. [1]

Medicinal plants are rich source of secondary metabolites. Secondary metabolites are present in small quantities in specialized cells, but they possess significant biological activities ranging from antibacterial, antibiotic, insecticidal, hormonal, pharmacological and pharmaceutical. [2]

Flavonoids are a group of about 4000 naturally polyphenolic compounds, found universally in plant origin. According to the differences in functional groups and their relative positions of the 15-carbon skeleton (aglycons), flavonoids are classified into several subgroups including the following: flavone, flavanone, flavonol, isoflavonoid, anthocyanidin, and chalcones. [3] They are generally not synthesized by the animals. An important effect of flavonoids is the scavenging of oxygen- derived free radicals. *In vitro* experimental systems also showed that flavonoids possess antiinflammatory, antiallergic, antiviral, and anticarcinogenic properties. [4].

The aim of this study was extraction and estimation of flavonoids from leaf extract of *Hyptis suaveolens* and to evaluate its *in vitro* antioxidant potential.

MATERIALS AND METHODS

Collection of plant material

The young leaves of *Hyptis suaveolens* were collected from Surat, Gujarat in month of December 2017, and authenticated by experts. The fresh leaves of plant were thoroughly washed 2-3 times to remove adhering dust and impurities. The leaves were blotted on filter paper.

Flavonoid Extraction

Leaf of *Hyptis suaveolens* was collected; shade dried, finely powdered. Finely powdered sample (100 g) was soxhlet extracted with 80 % hot methanol (500 ml) on a water bath for 24 h and filtered. Using separating funnel the filtrate obtained was re-extracted successively with petroleum ether (fraction I), ethyl ether (fraction II), and ethyl acetate (fraction III). Fraction of petroleum ether was discarded due to being rich in fatty substances, where as fractions of ethyl ether and ethyl acetate were further analyzed for free and bound flavonoids, respectively. Using 7 % H_2SO_4, ethyl acetate fraction of the sample was refluxed for the hydrolysis for 2 h (for removal of bounded sugars) and again filtrate was extracted in separating funnel with ethyl acetate. Ethyl acetate extract thus obtained was washed with distilled water to neutrality. Ethyl

ether (free flavonoids) and ethyl acetate fractions (bound flavonoids) were dried in vacuum and weighed. [5]

Qualitative analysis of flavonoids

Ethyl ether and ethyl acetate fractions were separately applied 1 cm above the edge of the pre-coated silica gel 60 F 254 aluminum plates along with the standard reference compound Quercetin.

These pre-coated silica gel 60 F 254 aluminum plate was developed on solvent system comprising of methanol: chloroform in the ratio of 5:5 and observed under visible light after spraying with 2% Lead acetate. The R_f value was calculated.

Quantitative analysis of flavonoids

The total flavonoids were estimated by aluminium trichloride colorimetric method. [6] A solution (2ml)of Fraction(II,III) was mixed with a solution (2ml) of aluminumtri chloride ($AlCl_3$) in methanol (2 %).The absorbance was read at 415 nm after10 min against a blank sample consisting of a methanol (2ml) and with $AlCl_3$. Quercetin was used as reference compound to produce the standard curve, and the results were expressed as mg of Quercetin equivalents (QE)/g of dry weight.

DPPH Free Radical Scavenging Assay

The DPPH antioxidant assay was determined as described by Shimada *et. al.*., 1992. [7] Briefly, 1 mM DPPH in 99.5% Ethanol. To 0.5ml of DPPH radical solution, Add 2 ml of the free and bound flavonoids solution, and the reaction mixture is vortexed for 10s and allow to stand at room temperature for 30 min. The absorbance is recorded at 517 nm by using UV- Spectrophotometer. Compare with the 75% ethanol which acts as control solution. Ascorbic acid is used as reference antioxidant compound.

The percentage of DPPH radical scavenging activity is expressed as:

DPPH scavenging effect (%) = [1- (Test sample absorbance/blank sample absorbance)] ×100(%).

RESULT AND DISCUSSION

Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress, which has been implicated in the pathogenesis of various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. [8]

Qualitative analysis of flavonoids

With the help of thin layer chromatography technique, both Ethyl ether and ethyl acetate fractions (bound and free flavonoids fraction) was compared with standard Quercetin. After development of chromatogram and by comparing R_f values of test compound with standard, it can be interpret that as the R_f value of bound flavonoids fraction matched with standard it might be Quercetin, and R_f value near around standard of free flavonoids fraction confirms the presence of flavonoids in plant. $R_f$value of respective compounds were listed in following table 1.

Table: 1 TLC analysis for Flavonoids and its $R_f$value.

Compound	R_f value
Quercetin	0.85
Bound Flavonoids (Ethyl acetate fraction)	0.85
Free flavonoids (Ethyl ether fraction)	0.83

Fig 1: TLC analysis with solvent system methanol: chloroform (5:5 ratio)
Quercetin Bound Flavonoids Free Flavonoids

Quantitative analysis of flavonoids

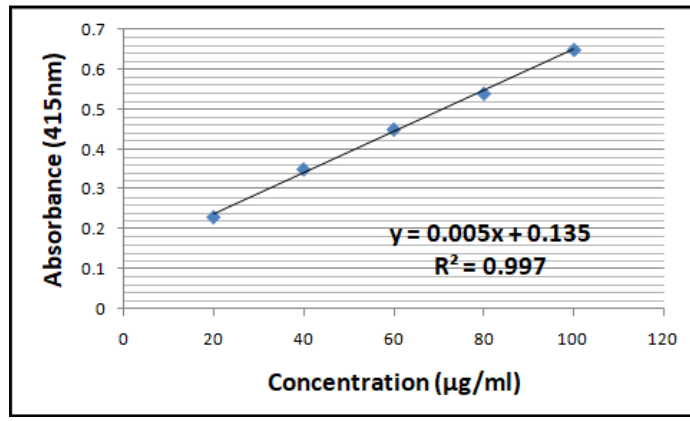

Fig 2: Standard curve of Quercetin.

Quercetin was used as a standard compound and the total Flavonoids were expressed as mg/g Quercetin equivalent (mg QE/gm) using the standard curve equation: $y = 0.005x + 0.135$, $R^2 = 0.997$, Where y is absorbance at 415 nm and x is total Flavonoid content. (Fig. 2)

In present study, total flavonoids from bound flavonoids fraction was found to be 20 mg QE/gm and from free flavonoids fraction was found to be 8mg QE/gm.

The qualitative and quantitative analysis confirms that plant used in this study *Hyptis suaveolens* is a rich source of flavonoids.

DPPH Free Radical Scavenging Assay

The free radical scavenging activity of flavonoids fractions was studied by its ability to reduce the DPPH. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. [9] A freshly prepared DPPH solution is of deep purple colour with absorption maximum at 517 nm and in the presence of antioxidant this colour disappears due to quenching of DPPH free radicals and convert them into a colorless product i.e. 2,2-diphenyl-1-hydrazine. Antioxidant mechanism performed by providing hydrogen atoms or electron. [9] DPPH radical scavenging ability was found 81% in free flavonoids fraction while 85% in bound flavonoids fraction.

CONCLUSION

Flavonoids have antioxidant powers that may provide important health benefits and generally not synthesized by animals so its uptake is essential. In this study it was found that *Hyptis suaveolens* is a rich source of flavonoids, and this can be use to extract dietary flavonoids.

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