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PHYTOCHEMICAL SCREENINGS AND PARTIAL CHARECTERIZATION OF *OCIMUMBASILICUM* PHENOLIC EXTRACT BY TLC

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ABSTRACT

The aim is to analysis and study the phytochemical constituents present in *Ocimumbasilicum* leave. Preliminary Phytochemical analysis of aqueous methanol extract of *Ocimum basilicum* leaves revealed the presence of bioactive constituents such as alkaloids, anthraquinones, coumarins, flavonoids, saponins, phlobatannins, tannins and terpenoids. These constituents were separated away on the basis of polarity of different solvents in to different fractions. These may be used in nutraceuticals and the food industry. However, additional studies are necessary to develop a method for the fractionation and identification of polyphenols and to determine the most active antioxidant compounds in the polyphenolic extract.

KEYWORDS: *Ocimumbasilicum* leave, phytochemical screening, medicinal uses and TLC.

INTRODUCTION

Traditional medicinal plants has focused on the discovery of valuable drugs during the past few decades (Buenz *et al*., 2004). Polyphenols are naturally occurring compounds found largely in the fruits, vegetables, cereals and beverages. Fruits like grapes, apple, pear, cherries and berries. Typically a glass of red wine or a cup of tea or coffee contains about 100 mg polyphenols. Cereals, dry legumes and chocolate also contribute to the polyphenolic intake (Scalbert *et al*., 2005 and Spencer *et al*., 2008). Polyphenols are secondary metabolites of

plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens (Beckman, 2000).

Distribution of phenolics in plants at the tissue, cellular and sub cellular levels is not uniform. Insoluble phenolics are found in cell walls, while soluble phenolics are present within the plant cell vacuoles (Adlercreutz and Mazur, 1997). Certain polyphenols like quercetin are found in all plant products; fruit, vegetables, cereals, fruit juices, tea, wine, infusions etc., whereas flavanones and isoflavones are specific to particular foods. In most cases, foods contain complex mixtures of polyphenols.

The outer layers of plants contain higher levels of phenolics than those located in their inner parts (Simon *et al*., 1992). Numerous factors affect the polyphenol content of plants, these include degree of ripeness at the time of harvest, environmental factors, processing and storage. Polyphenolic content of the foods are greatly affected by environmental factors as well as edaphic factors likesoil type, sun exposure, rainfall etc. The degree of ripeness considerably affects the concentrations and proportions of various polyphenols (Manach *et al*., 2004). Many polyphenols, especially phenolic acids, are directly involved in there sponse of plants to different types of stress: they contribute to healing by lignification of damaged are as possess antimicrobial properties, and their concentrations may increase after infection (Parr *et al*., 2000).

Role of Poly phenols

Poly phenols are a group of secondary metabolites substances in plants are usually sub divided into two groups: flavonoids and non flavonoids. The most common flavonoids in plants are flavonols (quercetin, kaempferol, and myricetin), flavan-3-ols (catechin, epicatechin, and tannins), and anthocyanins (cyanin). Non flavonoids comprise stilbenes, hydroxyl cinnamic acids and benzoic acids (Arnous, *et al*., 2002).

Phenolic acids, flavonoids and tannins are the most commonly found polyphenolic compounds in plant extracts (Naik*et al*., 2006). Flavonoids are 15-carbon compounds generally distributed throughout the plant kingdom (Harborne, 1988). Flavonoids and many other phenolic compounds of plant origin have been reported as scavengers of reactive oxygen species (ROS), and are viewed as promising therapeutic drugs for free radical pathologies (Parshad*et al*., 1998; Chang *et al*., 2007).

Tannins are naturally occurring, high molecular weight polyphenols which can be divided into hydrolysable tannins and condensed tannins. Tannins are the most abundant antioxidants in the human diet and they exhibit many biologically important functions which include protection against oxidative stress and degenerative diseases.

MATERIALS AND METHODS

Sterilization of Glassware

Glassware were soaked overnight in cleaning solution and washed thoroughly with running tap water. They were then cleaned with detergent solution and rinsed several times with tap water and finally in distilled water and air dried. The glassware and media were sterilized in an autoclave at 15psi for 20 minutes, at120^{o}C.

Preparation of extracts

Organic solvents (methanol) extract of the *Ocimum basilicum* leaf were prepared according to the method described by Boaky- Yiadon (1979) with little modifications. Twenty grams of *Ocimum basilicum* leaf extract were air-dried, crushed and blended into powder using an electric blender for each solvent. The blended material was transferred to a beaker and soaked separately in 100 ml of the organic solvent at room temperature. The mixture was extracted by agitation on arotary shaker. The extract obtained was vaccum-dried and used for further test.

Reagent

bismuth nitrates, Potassium iodide, Hcl, and Mercuric chloride.

Na OH solution, 10% lead acetate solution, chloroform, and concentrated Sulphuric acid.

FeCl3 (1%) and $K_3(Fe(CN)_6)$.

glacial acetic acid

ferric chloride solution.

olive oil

Total phenolic content of *Ocimum basilicum* leaf methanol extract

The total phenolic content of *Ocimum basilicum* leaf methanol extracts was determined using the method by Gutfinger (1981). The DSM extract (1 mL, 1mg/mL) was mixed with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2%Na2CO3, and centrifuged at 13400 X g for 5 min. The absorbance of upper phase was measured using a spectrophotometer (Model UV-

1601; Shimadzu, Tokyo, Japan) at 750 nm after 30 min incubation at room temperature. TPC was expressed as a tannic acid equivalent.

The Partial characterization of Thin Layer Chromatography in *Ocimum basilicum* leaf

The flavonoid fraction of *Ocimum basilicum* leaf was loaded on to pre coated TLC (60 F2 54) and it was developed using solvent system in the ratio of 1:0.5:0.1(Hexane, Chloroform and Methanol) visible and the non-visible spot given and it is fluorescent with UV light at 360nm.

RESULTS AND DISCUSSION

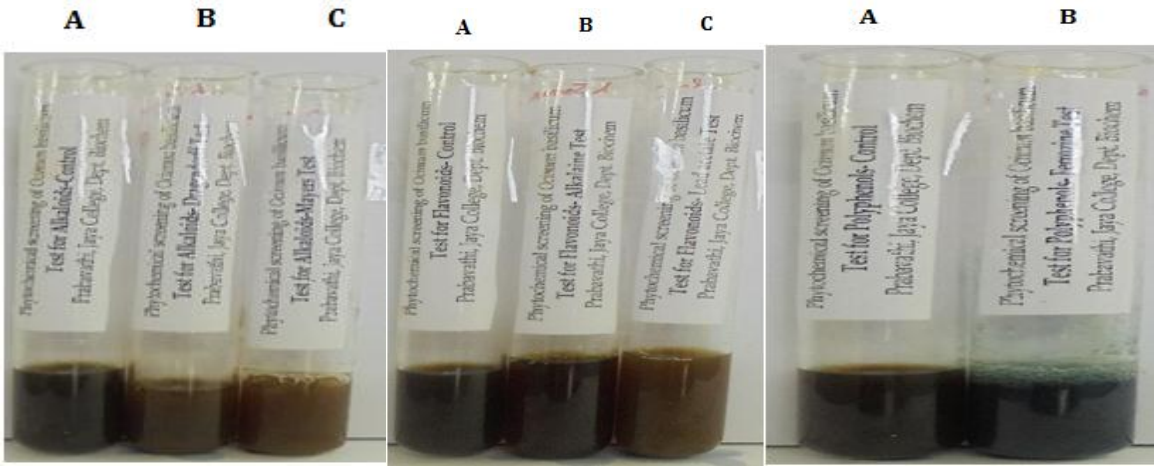
Phytochemical screening provides basic information about medicinal importance of a plant extract. In this study evaluation for qualitative and quantitative estimation of the chemical constituents of *Ocimumbasilicum* extracts showed the presence of various secondary metabolites. Phytochemical analysis of aqueous methanol extract of *Ocimum basilicum* leaves revealed the presence of bioactive constituents such as alkaloids, anthraquinones, coumarins, flavonoids, saponins, phlobatannins, tannins and terpenoids. These constituents were separated away on the basis of polarity of different solvents in to different fractions. The biochemical investigation reports indicated the same composition of phyto chemicals for the crude methanol extract of different plants (Sahreen *et al*., 2010; Sahreen *et al*., 2011).

The phyto chemical screening of the *Ocimum basilicum* extract were studied presently showed the presence of flavonoids, poly phenols, and saponins (Table -1 and Figure-1).

Table: 1 Phytochemical screenings of aqueous extract of *Ocimum basilicum*

Sl. No.	Phytochemical Constituents	Observation	Aqueous extract of *I.suffruticosum*
1	**Alkaloids** -Dragendorff's Test -Mayers test	 Orange / red precipitate	 - -
2.	**Flavonoids** -Alkalai Reagent -Lead acetate test	 Intense yellow colour Precipitate formed	 + +
3.	**Glycosides** -Keller-Killiani test	 Reddish brown colour ring formed	 -
4.	**Tannin** -$FeCl_3$ test	 Blue black coloration	 +
5.	**Saponins** -Frothing test	 Foam	 +
6.	**Terpenoids** *-Salkowski test*	 Dark reddish brown color	 +

		in interface	
7.	**Polyphenols** -Ferrozine test	Raddish blue	+



Test for Alkaloids

A- Control;

B- Dragendroffs Test;

C- Mayers Test

Test for Flavonoids

A- Control;

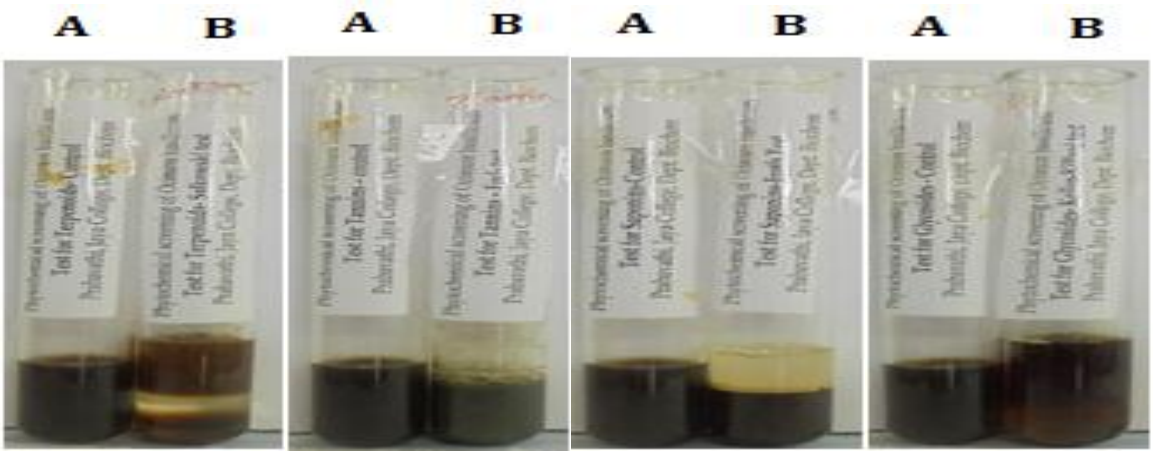
B- Alkali Reagent Test

C- Lead acetate Test

Test for Polyphenol

A- Control;

B- Ferrozine Test



Test for Terpenoid

A-Control;

B- Salkowiski Test

Test for Tannin

A- Control;

B- $Fecl_3$ Test

Test for Saponin

A- Control;

B- B- Froth Test

Test for Glycosides

A- Control; B- Keller-kilani Test

Figure: 1. Phytochemical screenings of aqueous extract of *Ocimumbasilicum*

The Partial charecterization of *Ocimum basilicum* by TLC

The phenolic extract of *Ocimum basilicum* loaded on Pre-coated TLC plates (60 F_2 54 Merck) and developed with a solvent system of Diaxon, tetra hydro furan and acetone in the

ratio of 6:6:1were efficient to extract the anti-inflammatory compound it is used for further studies. The developed plate was viewed under UV 240nm and 360nm (Table-2 and Figure-2).

Table: 2. Partial charecterization of *Ocimumbasilicum*phenolic extract by TLC

S.No	*Ocimum basilicum* phenolic extract		
	UV 240nm	UV 360nm	Visible
1.	0.32	0.32	-
2.	-	0.69	-
3.	-	0.80	-
4.	-	0.91	-

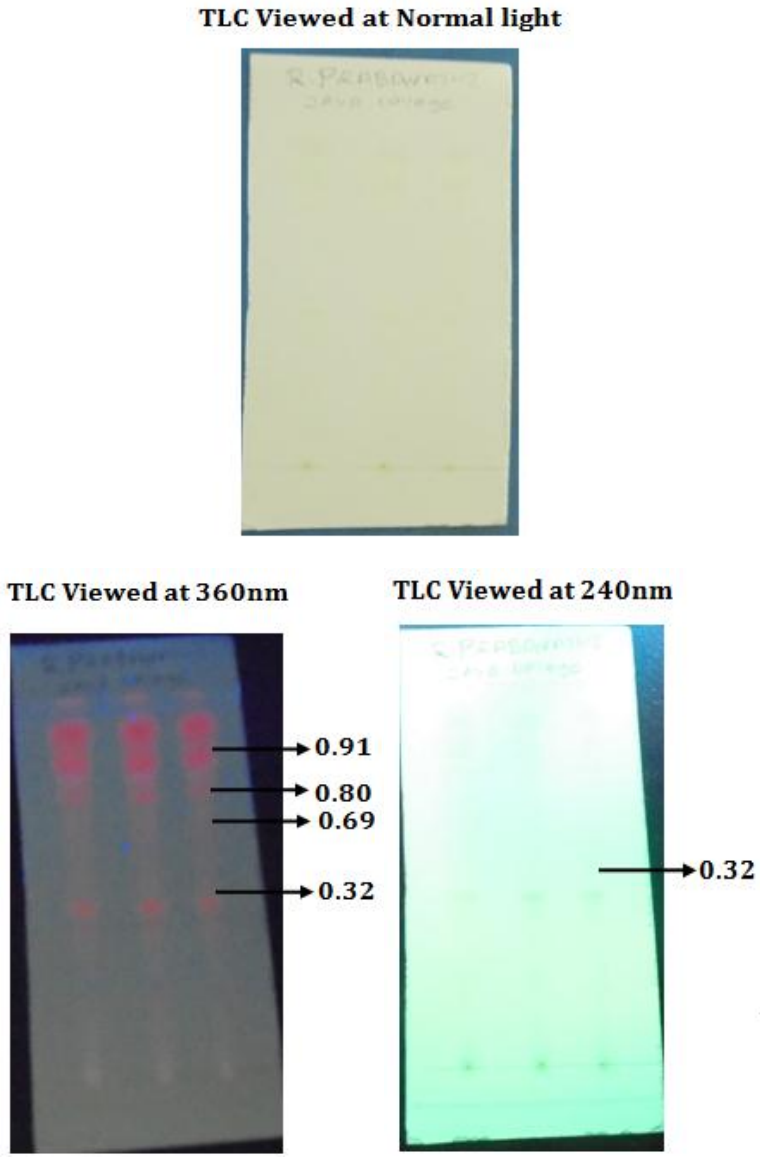


Figure: 2. Partial charecterization of *Ocimumbasilicum* phenolic extract by TLC

Total phenolic content of leaf extract of *Ocimumbasilicum*

In this context, the preliminary experiments revealed that 80% methanol was the best solvent for the extraction of phenolics from *Ocimum basilicum* at 60 °C for 60 min since it afforded a maximum yield of phenolics. The yields leaf of *Ocimum basilicum* extracts ranged from 29 % (w/w). Therefore, the total phenolic contents were reported as catechin equivalents (Figure-3 and Table-3).

Table: 3. Yield and phenolic content leaf of *Ocimumbasilicum*

Sample	Yield of extract (g/100 g of defatted CONTENT)	Total phenolic content (mg catechin equivalents per gram methanol extract)
leaf phenolic exctract of *Ocimumbasilicum*	29.1±1.5[a]	96.2±1.3[b]

[a] Data are expressed as mean ± standard deviation (n = 3) on a fresh weight basis.

[b] Means in each column sharing the same letter are not significantly (P = 0.05) different from other.

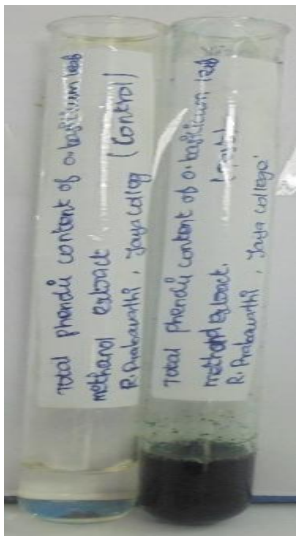


Figure: 3. Total phenolic content of leaf extract of *Ocimumbasilicum*

CONCLUSION

Polyphenols are valuable plant constituents for the scavenging of free radicals because of their phenolic hydroxyl groups. This, together with the obtained results, suggests that as the amount of polyphenolic compounds increases, the antioxidant activity also increases. In conclusion, the present study demonstrates that the polyphenolic extract of *Ocimumbasilicum* leave can be used in nutraceuticals and the food industry.

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