International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.9, No.4, pp 215-219, 2016

Phytochemical Analysis and Total Phenolic and Flavonoid Contents Determination of Methanolic Extract of *Ocimum basilicum* L seed

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Abstract : Seed of *Ocimum basilicum* L. belong to family Lamiaceae commonly known (Takhmaria) which has been used as traditional medicine in all asian country. Preliminary phytochemical analysis was done using various solvent extract. Aim of this study to determine total phenolic and flavonoid content of methanolic extract of *Ocimum basilicum* seed. Defatted powdered materials of *Ocimum basilicum* seed were extracted with Methanol. The level of phenolic and flavonoid content were determined by Folincoicalteu method and Aluminum chloride colorimetric method with gallic acid and rutin as standard. Determination of phenolic content by 7.15 ± 0.15 (mg GAE/ g extract), while flavonoid content determined by colorimetric method $AlCl_3$ is 3.28 ± 0.27 (mg RE /g extract).
Keywords: Flavonoid Content, Ocimum basilicum, Phytochemical Analysis, Total Phenolic Content.

Introduction:

Ocimum basilicum seed (Takhmaria /subja /sweet basil), family Lamiaceae most widely used in drinks in India, and Asian countries such as arabic falooda or sherbet. Seed of plant shows antimicrobial activity, in-vitro antioxidant, aphrodisiac, diuretic and anti dysenteric actions[1, 2] basil seeds were used to relieve indigestion, sore throat, constipation, diarrhoea seeds slow down the body process of converting carbohydrates into sugars, also contributing to weight loss and appetite. That accounts for the feeling of fullness lasting longer and can be useful for both weight loss and diabetes. The mucilaginous gel that forms around the seeds when they are soaked acts as an emollient, which soothes mucous membranes. It can be used to relieve constipation and diarrhea, appetite suppressant during weight loss programs, when eaten (or drunk) before meals and also consumed during the summer time, as it is one of the best body coolant.[3]

Traditionally, it is used for the treatment of pains and respiratory tract infections diabetes, asthma and decrease platelet aggregation[3].

D-xylose, L-arabinose, r-rhamnose, and D-galacturonicacid, galactose, glucose were isolated from seed of *Ocimum basilicum*[4]. Presence of the other compounds reported from the ethanolic extract of seed includes alkaloids, flavonoids, amino acid, protein, saponins, fat and oil[5].

In addition, secondary metabolites of plants that contain phenolic and flavonoid has been reported have some pharmacological activities, e.g antioxidant, antibacterial, antiviral, anti diabetic, anti inflammatory, anti-allergic, and anticancer, neurodegenerative and vasodilatory effect[6].

Based on above information, it needs further exploration for instance determination of phenolic and flavonoid content of methanolic extract of *Ocimum basilicum* seed in order to develop the potency of this plant become traditional medicine.

Materials and Methods

Material

Powder of *Ocimum basilicum* seed, Methanol, Folincoicalteu reagent, Sodium carbonate, Gallic Acid, Rutin, Aluminum chloride, Potassium acetate

Extraction of *Ocimum basilicum* seed

Air-dried powdered (100 gm) of *Ocimum basilicum* seed samples were defatted by refluxing with 250ml petroleum ether (60-80 °C) for 4 hrs. The residue was dried and then subjected to extraction by soxhlet method using different polarities of the solvent. The flask was refluxed for 12 hr and filtered through whatman filter paper. This procedure was repeated three times to get complete extraction from powder. Powder extracts were combined and evaporated to rotary evaporator to get the residue.

Phytochemical screening

The extracts prepared were analyzed for the presence of various phytoconstituents such as alkaloids, phenolic , flavonoids, saponin, tannins, steroids, anthraquinone, cardiac glycosides and reducing sugars based on the protocols available in the literature[7- 10.]

Preliminary Phytochemical analysis, was done using various solvent viz. hexane, ethyl acetate, chloroform, methanol of *Ocimum basilicum* seed screening for alkaloids (Mayer's test), saponins (foam test), tannins(lead acetate), phytosterol(Salkowski test), phenols (Ferric Chloride test),protein(Biuret test), flavonoids (Alkali test), triterpenoids (Liebermann – Burchard test) , carbohydrate (Molisch's test) amino acid and glycosides(Legal's test), were carried out.[12]

Determination of total phenolic content:

The content of total phenol in methanolic *ocimum basilicum* seed extracts were determined spectrophotometrically using Folin–Ciocalteu reagent[13] with modifications. The stock solution of gallic acid (100µg/ml) was prepared in methanol. From the stock solution of standard 0.1,0.2,0.3,0.4,0.5&0.6 ml were taken which gave 1,2,3,4,5 & 6 µg/ml concentration respectively was taken in 10 ml volumetric flask. To this 4 ml water and 0. 6 ml of folincoicalteu reagent was added. The above mixture was kept for 5 minutes and then 1.6 ml of 20% sodium carbonate solution was added and volume made up to 10 ml with distilled water. This mixture was kept for 30 minutes and the absorbance of blue color developed was measured at 765 nm. Total content of phenolic in the plant extracts were expressed as gallic acid equivalents (mg of GAE/g sample) and were calculated by the formula:

$T = (C \times V) / M$ Where,
T=total content of phenolic compounds (mg of GAE/g sample)
C=the concentration of gallic acid established from the calibration curve (mg/ml)
V= volume of extract (ml)
M= weight of methanolic plant extract (gram)

Determination of total flavonoid content:

The total flavonoid in the crude extracts was measured using the Aluminum Chloride Colorimetric Method[14]. Standard curve was prepared using Rutin as standard. The stock solution of rutin (100µg/ml) was prepared in methanol. From this solution 0.1,0.2,0.3,0.4, 0.5,& 0.6 ml taken and diluted up to 10 ml with

methanol to produce 1,2,3,4,5, & 6 µg/ml concentration respectively The standard solution were separately mixed with 1.5 ml of methanol, 0.1 ml 10% aluminum chloride, 0.1 ml 1 M potassium acetate and 2.8 ml distilled water. After incubation at room temperature for 30 minutes the absorbance of reaction mixture was measured at 415 nm. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank.

Total content of flavonoid in the plant extracts were expressed as rutin equivalents (mg of RE/g sample) and were calculated by the formula

$T = (C \times V) / M$ Where,
T = total content of flavonoid compounds (mg of RE/g sample)
C = concentration rutin established from the calibration curve (mg/ml)
V = volume of extract (ml)
M = weight of methanolic plant extract (gram)

Result and Discussion

Phytochemical analysis:

Preliminary phytochemical analysis revealed the best result in methanolic extract. Methanolic seed extract of *Ocimum basilicum,* showed the presence of Carbohydrate, Saponins, Phenolics, flavonoids, and Seed mucilage. (Refer Table: 1)

Table: 1 Phytochemical Analysis of *Ocimum basilicum* seed

Test	Hexane	Ethyl Acetate	$CHCl_3$	Methanol
Alkaloids	+	+	_	_
Carbohydrates	_	_	+	+
Phytosterols and triterpenoids	+	_	_	_
Saponins	_	_	_	+
Phenolics	_	_	+	+
Protein	_	_	+	
Flavonoids	_	_	_	+
Fat and oil	+	+	_	_
Seed mucilage	_	_	_	+

+Present - Absent

The determination of phenolic content by folin-ciocalteau method with gallic acid as standard with linear regression (Figure 1), with amount of phenolic was 7.15±0.15 (mg GAE/ g extract) (Table 2).

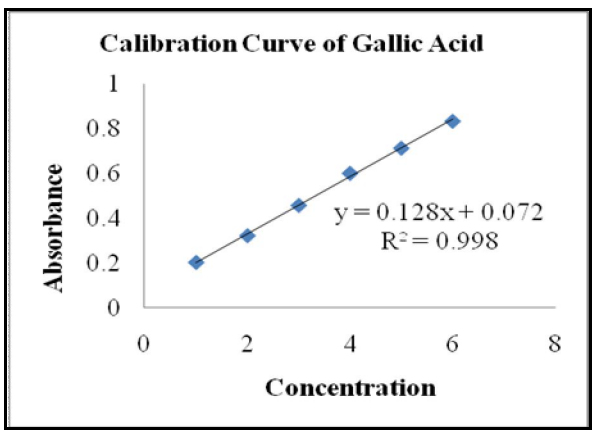

Figure 1 calibration curve of Gallic acid.

Table 2: Total phenolic and flavonoid contents of the methanolic seed extract of *Ocimum basilicum*

Plant part	Total Phenolic content (mg GAE/ g extract)	Total Flavonoid content (mg RE /g extract)
Seed	7.15 ± 0.15	3.28 ± 0.27

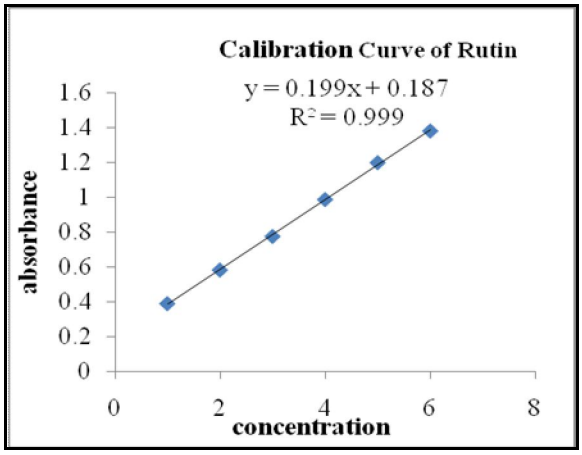

Figure 2 calibration curve of Rutin.

The determination of flavonoid total of methanolic extract of (*O.basilicum)* seed with rutin as standard curve (figure 2) with amount of flavonoid was 3.28±0.27 (Table 2). The seed of *Ocimum basilicum* were collected from the local market, Ahmedabad, Gujarat. & were authenticated by Dr. Bhasker L Pungani (Head P.G Center in Botany, Smt.S.M.Panchal Science College, Talod, Gujarat). Seed were powdered in order to extract more chemical compounds. *Ocimum basilicum* seed samples were defatted by refluxing with petroleum ether (60-80 °C) for 4 hrs in order to remove oily compound. The residue was dried and then subjected to extraction by soxhlet method using different polarity of solvent.

The potency of extracts to be traditional medicine depends on their chemical compounds (secondary metabolite). Phenolic and flavonoid occur widespread in some plants. Some studies have been reported the biological activities of those compounds. Thus, the determination of phenolic and flavonoid total is need to conduct.

Phenolic that occurs in extract is analyzed by using spectrophotometric UV-Vis[15]. Folin-Ciocalteau causes bathochromic wavelength movement around 765 nm which is showed by the color change from yellow to blue after incubated. Its color change occurs due to the reduction which leads to make complex between phenolic group and the tungsten and molibdat.

Then it is plotted to curve (concentration *vs* absorption) so as to obtain linear regression y = 0.1284x +0.0722 and yield R^2 = 0.9988 which indicates good linearity. Subsequently, it then be used to determine of phenolic level of sample compare and the yield is 7.15 ± 0.15 (mg GAE/ g extract)

Flavonoid content analyzing by colorimetric method with rutin as a standart, the one of flavonoid compounds that occurs in numerous plants, as comparison. For measuring flavonoid total, it needs to add 10 % $AlCl_3$ into extract solution in order to lead to complexity form between flavonoid and $AlCl_3$ which indicates with color changing into yellow at maximum wavelength 415 nm. Aluminum chloride forms stable complexes with C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid liable complexes with ortho-dihydroxyl groups in the A or B-ring of flavonoids.

The yield of absorption of standard rutin then was plotted to curve of rutin concentration (x) and absorption (y) and was obtained linear regression y = 0.1997x + 0.1872 with R^2 = 0.9995, the R^2 value shows the good linearity. Therefore, it can be used then to determine the level flavonoid total 3.28±0.27 (mg RE/g extract).

Conclusion:

From above study conclude that *Ocimum basilicum* seed containing higher amount of phenolic as well as flavanoid content. Present study confirms the use of *Ocimum basilicum* seed in traditional medicines and phytochemical data will be helpful in the standardization and quality control of precious indigenous drug and also for pharmaceutical industries.

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