International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 758-762
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Protein Precipitant Activity of *Ocimum basilicum*

Venkatarao Vutla[1]*, B. Kusuma Sowjanya[1], D. Tejaswini[1], E. Indumathi[1], J. Hari Sreekanth[1], T. Dileepkumar[1] and Suryadevara Vidhyadhara[1]

[1]Department of Pharmacognosy and Phytochemistry, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur, Andhra Pradesh - 522019.

Received: 12 Mar 2019 / Accepted: 14 Apr 2019 / Published online: 1 Jul 2019
***Corresponding Author Email:** vvrao_pharma@yahoo.co.in

Abstract

Tannin fractions were isolated from Leaves of *ocimumbasilicum*, used tannin acid as standard. The total phenolic compound and were determined. It was found that the tannins from the leaves of tulasi had the highest total phenolic compound and the lowest hydrolysable tannins. The interaction between tannins fraction from plant sources and tannic acid (as standard) with casein precipitated from skim milk (by acidification) was determined using precipitating potential method. The optimum pH of the interactions was at pH 5, while the lowest interaction was at pH 7. The highest precipitating potential was noted for tannic acid followed by tulasi leaves. Moreover, the tannins-casein complex was determined using fluorescence quenching method. The interaction between casein and tannic acid had the most extensive fluorescence quenching for plant tannins.

Keywords

Tannins, Interaction, Casein and Fluorescence quenching.

*****

INTRODUCTION:

Tannins are polyphenols that occur widespread in plant-based food. They are formed as secondary metabolites in plants and include a wide range of the algometric and polymeric poly phenols. Hydrolysable tannins (proanthocyanidins) are the most widely occurring tannins (Frazier *et al* 2010). In recent years, tannins production has become a very important issue because of their increasing commercial interest in the field of pharmaceutical, food and nutraceutical industries (Capparucci*et al* 2011). Moreover, tannins have beneficial affects by acting as antioxidants (Soares *et al* 2007). Nowadays, pulses are gaining more interest in the field of developing healthy and functional foods. Tulasi plant (Ocimumbasilicum.) is a member of the lamiaceae or labiataea family and constitutes one of the most important traditional dietary components (Faris *et al* 2012). It is rich in polyphenols and antioxidants and use as food on nutritional supplements in many traditional diets throughout the world (Talukdar 2013). Tulasi (*Ocimumbasilicum)*, contain antioxidant and antiflammatory compounds including more than a twelve phenolic acid, numerous tannins and wide variety of flavanoids which help explain the decrease risk of certain cancers in relationship to walnut consumption. Tulasi Leaves has been regarded as a rich source of flavan 3-ols also known as catechins, tulasi leaves is one major dietary source of polyphenols, (Haratifar 2012).

PLANT PROFILE:

Basil also called great basil or Saint-Joseph's-wort, is a culinary herb of the family Lamiaceae (mints). Basil is native to tropical regions from central Africa to Southeast Asia. [3] It is a tender plant, and is used in cuisines worldwide. Depending on the species and cultivar, the leaves may taste somewhat like anise, with a strong, pungent, often sweet smell. There are many varieties of basil, as well as several related species or hybrids also called basil. The type used commonly as a flavor is typically called sweet basil (or Genovese basil), as opposed to Thai basil (O. basilicum var. thyrsiflora), lemon basil (O. × citriodorum), and holy basil (Ocimumtenuiflorum). While most common varieties of basil are treated as annuals, some are perennial in warm, tropical climates, including holy basil and a cultivar known as "African blue basil".

Fig1. Ocimum Basilicum Whole plant

MATERIALS AND METHODS:

Collection of plant materials: The leaves of *Oscimum basilicum* were collected from the medicinal garden of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences and authenticated by Department of Botany and Microbiology, Aacharaya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh.

Extraction of Ocimum basilicum leaves: Fresh leaves of Ocimum basilicum were collected and extracted with ethanol & water (70:30) as solvent by using maceration for 14 days. Then the extract was further concentrated and the residue was stored in refrigerator. Subsequently, the mixture was filtered and concentrated under nearly vacuum pressure and at 40°C using rotary evaporator. The concentrated methanol extracts were further subjected to partial fractionation with solvents of increasing polarity viz. Hexane: Chloroform: Ethyl acetate: Methanol. Four fractions of the crude extract, with different polarity through in-solution isolation and using the difference in various secondary metabolites' polarity were prepared. To isolate the hexane fraction, the extract concentrated, suspended in 80% ethyl alcohol, and mixed with equal volume of normal hexane (Merck, Germany) with shaking vigorously. The remaining solution from which the ethyl alcohol was removed was mixed with distilled water and with chloroform (Merck, Germany) in equal volume, shaken, and hydrated using sodium sulfate and used as the Chloroform fraction. Subsequently, remaining solution was mixed with ethyl acetate (Merck, Germany) in equal volume, shaken and used as the ethyl acetate fraction. To prepare n-Butanol fraction, equal volume of n-Butanol (Merck, Germany) was added to the remaining aqueous phase of the material, shaken and concentrated at 40°C and in vacuum condition. The remaining aqueous phase was concentrated, under the similar condition, as mentioned above and used as aqueous fraction. The fractions of hexane, chloroform, ethyl acetate, and n-butanol were collected individually and dried under vacuum below 45°C, while the residue was kept without any further treatment.

PHYTOCHEMICAL INVESTIGATION:

The plant aqueous, ethanolic, acetone and methanolic extracts were screened for the presence of the phytochemical classes by using the standard following methods.

ASTRINGENT ACTIVITY OF PLANT EXTRACT AND ITS FRACTIONS

The astringent activity of plant extract is also determined by fluorescence quenching method

Proteins used: Reference Protein: Gelatin (1mg/ml)

Sample protein: Casein - 1mg/ml

Reagents used:

Preparation of standard solution: A 1mg/ml standard solution of tannic acid was prepared I distilled water and made up to 2gm/ ml. From this stock solution different concentration of 5, 10, 15, 20, 25 micrograms/ml were taken for protein interaction studies.

Preparation of stock solutions of fractions: 2.5mg/ml Solutions of hydro alcoholic extract and

each of ethyl acetate, chloroform, n-hexane fractions was prepared and diluted appropriately.

Ascorbic acid was prepared in distilled water of different concentration such as 60, 120, 180, 240, 300, 360, 420, 480 µg/ml.

Sodium phosphate buffer of PH 6.2 to 7.0

Preparation of stock solution of buffer: Sodium di hydrogen phosphate 0.136 gm was dissolved in distilled water and make upto 100 ml.

PROCEDURE:

Fluorescence Quenching Method:

The interactions between tannin fractions and casein yielding soluble complexes were investigated using fluorescence quenching method, Soares et al. (2007). The fluorescence quenching involves a reduction in fluorophore fluorescence in the presence of quencher. All measurements were taken in quartz cuvette (1.0 X 1.0 X4.0 cm) using (**Perkin Elmer Luminescence Spectrometer 50B fluorescence spectrometer (Beaconsfield, Great Britain**). Fluorescence emission spectra were recorded in the wavelength range of 285–500 nm by exciting protein at excitation wavelength (λex) of 282 nm. The slit width for both excitation and emission was set to 5 nm. To determine the linear concentration, range for protein fluorescence, a series of tannins fractions and casein solutions with increasing concentration were prepared in 0.1M sodium phosphate buffer of pH 5. Suitable protein concentration was chosen for fluorescence quenching experiments. To 2 ml of protein solution (2 mg/100ml 0.1M sodium phosphate buffer pH 5) portions of 0, 10, 20, 30, 40 and 50µl of tannin fraction solution (2.5 mg/100ml 0.1M sodium phosphate buffer pH 5) were added and the mixture was shaken. The changes of fluorescence intensity were measured within 30s after addition. All fluorescence readings were corrected for protein dilution effect. The titration was performed in four replications. In order to avoid artefact quenching, tannin fractions solution was checked for its intrinsic fluorescence. All measurements were taken at room temperature.

RESULTS:

Table: 1 Preliminary Phytochemical screening for Hydro-alcoholic extract of Ocimumbasilicim

S.No	Plant Constituents	Test Performed	Result
1.	Carbohydrates	Molisch's test	+
2.	Proteins	Ninhydrin test	_
3.	Flavanoids	Lead acetate test	+
4.	Alkaloids	Mayer's test	+
5.	Steroids	Liebermann burchard's test	+
6.	Terpenoids	Salkowski test	+
7.	Saponins	Froth test	+
8.	Tannins	Ferric chloride test	+
9.	Glycosides	Keller- kiliani test	+
10.	Phenolic compounds	Ferric chloride test	+
11.	Fixed oils	Spot test	_

Table-2 UV Spectrophotometry of StandaradTannicAcid

S.NO	CONC (µg/ml)	ABSORBENCE
1	5	0.156
2	10	0.245
3	15	0.467
4	20	0.578
5	25	0.781

Table 3: UV spectrophotometry of Hydro alcoholic extract and solvent fractions of Ocimumbasilicum

Concentration (µg/ml)	Absorbance (A 700)			
	Hydro alcoholic extract	Ethyl acetate fraction	Chloroform fraction	N-Hexane fraction
5	0.2592	0.0016	0.0167	0.0187
10	0.2948	0.0044	0.0205	0.0207
15	0.2752	0.0072	0.0260	0.0280
20	0.3327	0.0091	0.0282	0.0312

Table 4: Total Tannin Content of Hydro Alcoholic Extract and Solvent fractions of Ocimumbasilicum

Concentration (µg/ml)	Total Tannin Content (mg TAE/g)			
	Hydro alcoholic extract	Ethyl acetate fraction	Chloroform fraction	N-Hexane fraction
5	287.8±12	243.9±13	189.8±14	154.4±12
10	345.15±15	267.8±17	196.09±19	176.09±16
15	367.8±17	279.08±18	205.9±13	180.45±13
20	398.06±12	292.87±19	213.3±14	193.8±23

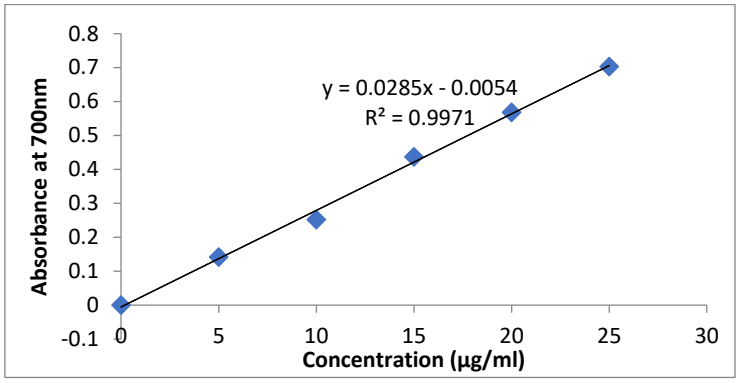

Fig3. Calibration Curve for Standard Tannic Acid

Table:5 Protein Precipitant Activity of Ocimumbasilicum leaves

S.NO	CONC(µg/ml)	INTENSITY
1	20	245.87
2	40	342.76
3	60	234.71
4	80	231.56

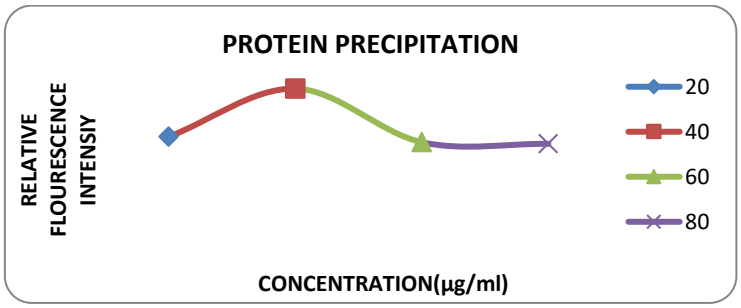

Fig. 8 Intensity Curve for protein precipitant activity

DISCUSSION OF RESULTS:

Tannins isolated from Ethyl acetate fraction had the highest phenolic content 425.87±05 mg tannic acid equivalent/g while tannins from N-hexane fraction had the lowest pheniloc compound 313.51±10 mg tannic acid equivalent/g. The interactions between all tannins fraction and casein were demonstrated to be pH dependent, these agree with that reported by Kosińska et al (2011). The maximum precipitation points (high A700 values) were at pH 5 for all complexes. Hagerman and Butler (1978) suggested that the strongest protein phenolic compounds interactions occurred at pH close to isoelctric point of protein. Neves et al (1997) revealed that casein precipitation was at pH 5 for all tannin-casein interactions.

The results were in order of ethyl acetate> Hydro alcoholic> chloroform>N-hexane tannins. Karomać et al (2007) reported that walnut tannins had more BSA precipitating activity than lentil tannins. This may be related to the low content of condensed tannins in walnut as mentioned before hence those hydrolysable tannins generally exhibit precipitating potential (Spencer et al 1988 and Haslam 1996).

CONCLUSION:

Plants of *Ocimum*species have great medicinal values for treating various health problems and were used throughout the world. Tulsi protects against diseases and reduces stress; enhances stamina and endurance; increases the body's efficient use of oxygen; Immune system; reduces inflammation; protects against radiation damage; lessens aging factors; supports the heart, lungs and liver; has antibiotic, antiviral and antifungal properties; Enhances the efficacy of many other therapeutic treatments; and provides a rich supply of antioxidants and other nutrients. Overall, tulsi is a premier adaptogen, helping the body and mind to adapt and cope with a wide range of physical, emotional, chemical and infectious stresses, and restore disturbed physiological and psychological functions to a normal healthy state. This general vitality enhances and health promoting properties, in addition to it has many more specific therapeutic actions, likely account for much of the exceptionally broad range of Tulsi's traditional medical uses. The interaction of tannins fraction isolated from Ocimum and its various solvent fractions with casein (milk protein) resulted an insoluble and soluble complexes formation. The extent of precipitation was depended on pH and the ration between tannins and casein. *O. basilicum* showed significant results in ethyl acetate extracts.

BIBLIOGRAPHY:

1. Agrawal P, Rai V, Singh RB. Randomized placebo-controlled, single blind trial of holy basil leaves in patients with noninsulin- dependent diabetes mellitus. *Int J Clin PharmacolTher*1996;34(9):406-9.
2. Arenal A, Martín L, Castillo NM, de la Torre D, Torres U, Gonza´lezR. Aqueous extract of *Ocimumtenuiflorum* decreases levels of blood glucose in induced hyperglycemic tilapia (*Oreo-chromisniloticus). Asian Pac J Trop Med 2012; 5(8):634-7*.
3. Banerjee S, Prashar R, Kumar A, Rao AR. Modulatory influence of alcoholic extract of *Ocimum*leaves on carcinogen induced metabolizing enzyme activities and reduced glutathione levels in mouse. Nutr Cancer 1996; 25:205-217.
4. Bihari CG, Manaswini B, Panda SKSP, Tripathy SKST. Phyto-of leafy induced diabetic model. *J Pharm Res* 2011; 4:28-9.
5. Chattopadhyay RR. Hypoglycemic effect of *Ocimum sanctum* leaf extract in normal and streptozotocin diabetic rats. *Indian J Exp Biol*1993; 11:891.
6. Casanova LM, da Silva D, Sola-Penna M, de Magalhães Camargo LM, de Moura Celestrini D, Tinoco LW, et al. Identifi- cation of chicoric acid as a hypoglycemic agent from *Ocimumgratissimum* leaf extract in a biomonitoring *in vivo* study. *Fito-terapia*2014; 93:132-41.
7. Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of diabetes and diabetes-related complications. Phys Ther2008;88(11):1254.
8. Dharmalingam, R and Nazni.P**,** Phytochemical Evaluation of *Coriandrum* L Flowers, International Journal of Food and Nutritional Sciences, 2013, Vol.2, Iss.4, (Oct-Dec) pp.35-39, e-ISSN 2320–7876.
9. Egesie UG, Adelaiye AB, Ibu JO, Egesie OJ. Safety and hypo-*glycaemic properties of aqueous leaf extract of Ocimum gratissi- mum* in streptozotocin induced diabetic rats. *Niger J PhysiolSci*2006; 21(1–2):31-5.
10. El-Beshbishy HA, Bahashwan SA. Hypoglycemic effect of basil (*Ocimum basilicum*) aqueous extract is mediated through inhibition of a-glucosidase and a-amylase activities: an in vitro study. *Tox-icolIndHealth* 2012;28(1) :42-50.