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Evaluating *Ocimum basilicum* and *Ocimum tenuiflorum* leaf extracts for the presence of phenolic compounds

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Abstract

Ocimum is the genus of aromatic herbs belonging to the family Lamiaceae. They are native to tropical and warm temperate climates of all the inhabited continents. The genus *Ocimum* includes several species of Basil in India, the most common being the *Ocimum basilicum* (Sweet Basil) and *Ocimum tenuiflorum* (Holy Basil). Both the species are known for their ornamental and therapeutic importance attributed to their various phytochemical constituents. The present study was undertaken to assess the qualitative phytochemistry and to estimate the total phenolic content of the two plant extracts by Spectrophotometry and Thin Layer Chromatography. The results thus obtained suggest that the leaves of *Ocimum basilicum* and *Ocimum tenuiflorum* are potential sources of healthy phytochemicals particularly phenols.

Keywords: *Ocimum*, phenolics, thin layer chromatography, spectrophotometry

Introduction

Medicinal plants are widely used by traditional healers for curing various diseases in humans and livestock. In the recent years, scientific evaluation of medicinal plants has gained more attention against conventional drugs due to their immense benefits. As medicinal plants produce several useful phytochemical compounds through primary or secondary metabolism, they can be used as an alternative to synthetic drugs. Phytochemicals play a major role in plant defence mechanisms against competitors, predators and pathogens. They are majorly classified into carotenoids and polyphenols which include phenolic acids, flavonoids, stilbenes and lignans (Molyneux *et al.*, 2007) ^[1].

Ocimum basilicum, commonly known as Sweet Basil, is a medicinal herb with pleasant fragrance possessing immense therapeutic and culinary applications. The aroma of each type of Basil is determined genotypically and it depends upon the combination of essential oils that varies with different countries (Rajesh, 2014) ^[2]. The essential oil of *Ocimum* contains many compounds like linalool, chavicol, eucalyptol, menthol, eugenol, cadinol, camphor, cinnamate, caryophyllene, elixen, gurjunene and cadinene (Zamfirache *et al.*, 2011) ^[3]. Some phenolic acids identified are present in the form of caffeic acid derivatives and flavonol-glycosides (Kivilompolo and Hyotylainen, 2007; Toussaint, 2007; Nguyen *et al.*, 2008) ^[4-6].

Ocimum tenuiflorum, commonly known as Holy Basil is one of most cherished herb, traditionally used in the Indian system of medicine for its diverse healing properties. It has a highly complex chemical composition containing many nutrients and biologically active compounds such as ursolic acid, carvacrol and limatrol. Other constituents include phenolic acids such as rosmarinic acid, propanoic acid, apigenin, cirsimaritin and water-soluble flavonoids namely orientin and vicenin (Shishodia *et al.*, 2003) ^[7].

Materials and Methods

Plant Source

Fresh leaves of *Ocimum basilicum* and *Ocimum tenuiflorum* were collected from Herbal Garden, Ethno Veterinary Herbal Product Research and Development Centre, TANUVAS, Orathanadu, Thanjavur District, Tamil Nadu. The leaves were cleaned, shade-dried and powdered for further analysis.

Preparation of Methanolic Extracts

25g of the powdered sample was extracted with 125ml of methanol for 6 hours in a Soxhlet apparatus (Uma *et al.*, 2009) ^[8]. The extracts were kept in desiccators to allow the solvent to evaporate completely.

Preliminary Phytochemical Screening

Phytochemicals present in the leaf extracts were screened as per standard protocols for the presence of phytochemical constituents such as alkaloids, glycosides, tannins, saponins, phenols, flavonoids, terpenoids and steroids.

Thin Layer Chromatographic Analysis

TLC was carried out on a 20x20 cm silica gel pre-coated aluminium sheets (Merck). 10 µl of each leaf extract was placed on the TLC plate at an equal distance. The extract-loaded plates were kept in a chromatographic chamber and allowed to run in chloroform: ethanol (7:3) solvent system (Jaliwala *et al.*, 2011) [9] for 10 minutes after which it was subjected to Iodine vapour in a glass beaker containing iodine crystals (Mathuravalli and Eswarakshmi, 2012) [10]. A change in the colour of the sample-run spots indicated the presence of phenols. The Retardation factor (R_f) which indicates the ratio of the distance travelled by the compound to the distance travelled by the solvent in a given time, was measured for both the sample spots.

Total Phenolic Estimation

The total phenolic content of the leaf extracts was measured with Folin-Ciocalteu's reagent method (Singleton *et al.*, 1999) [11] and expressed in terms of gallic acid equivalent (GAE). Gallic acid calibration solutions having final concentrations ranging from 2-10 µg/ml were used to plot a standard calibration curve as defined by Koc *et al.*, (2010) [12]. 750 µl of Folin-Ciocalteu's reagent was added to 100 µl of each extract and shaken well. After 5 min, 750 µl of (6%) sodium carbonate solution was added to the mixture. After incubation for 90 minutes at room temperature, the absorbance was determined at 765nm in an UV-visible spectrophotometer. The total phenolic content was determined using an equation obtained from the calibration curve and was expressed as µg GAE/ml (Benedec *et al.*, (2012) [13] and Gayosa *et al.*, (2004) [14].

Results and Discussion

The results of the preliminary phytochemical screening of methanolic leaf extracts of *Ocimum basilicum* and *Ocimum tenuiflorum* as summarized in Table 1 revealed the presence of phenols, glycosides, flavonoids, terpenoids and saponins. A similar results was earlier given by Srinivas *et al.*, (2015) [15]. Other reports by Sanni *et al.*, (2008) [16] and Daniel *et al.*, (2011) [17] showed the presence of alkaloids, tannins and steroids from minute to rich quantities. As suggested by Jirovetz *et al.*, (2003) [18], this dissimilarity in the qualitative phytochemical composition may have resulted due to different environmental conditions and agronomical practices followed.

The estimation of total phenolics by spectrophotometry as shown in Table 2 revealed that *Ocimum basilicum* and *Ocimum tenuiflorum* were found to contain 30.47 mg GAE/g sample and 50.89 mg GAE/g sample of total phenolic content, respectively. Conversely, earlier reports by Gajula *et al.*, (2009) [19], Rafat *et al.*, (2010) [20] and Benedec *et al.*, (2012) [13] showed varying concentrations of total phenolics in leaf extracts of *O. basilicum* and *O. tenuiflorum* sourced from Malaysia, Denmark, Cuba and Romania. The quantitative divergence in the total phenolic content of *Ocimum spp* may be due to variations in geographical, climatic and soil conditions existing in different parts of the world. This in turn may affect the chemical composition and secondary metabolites of the plant (Vina and Murillo, 2003) [21].

The results of Thin Layer chromatographic analysis are presented in Table 3, Fig 1. TLC developed in the mobile phase of chloroform: methanol: acetic acid for methanolic extracts of *O. basilicum* and *O. tenuiflorum* with gallic acid as the reference compound showed R_f values of 0.68 and 0.69 respectively, compared to the gallic acid standard of 0.7, after exposure to iodine vapour. Earlier reports by Om prakash *et al.*, (1998) [22] and Kondawar *et al.*, (2011) [23] stated that gallic acid showed varying R_f values in different solvent systems.

Table 1: Preliminary Phytochemical Screening of methanolic leaf extracts of *Ocimum basilicum* and *Ocimum tenuiflorum*

Phytochemical compound	Method	<i>Ocimum basilicum</i>	<i>Ocimum tenuiflorum</i>
Alkaloids	Dragendorff test	-	-
Glycosides	Keller Killani Test	+	+
Tannins	Ferric chloride test	-	-
Saponins	NaOH test	+	+
Phenolics	Ferric chloride test	+	+
Flavonoids	Shinoda Test	+	+
Terpenoids	Noller Test	+	+
Steroids	Salkowski Test	-	-

Table 2: Estimation of Total Phenolic content by Spectrophotometry

Plant	TPC (mg GAE/g of sample)
<i>Ocimum basilicum</i>	30.47
<i>Ocimum tenuiflorum</i>	50.89

Table 3: Thin Layer Chromatographic Analysis

Plant	Retardation factor (R _f)
<i>Ocimum basilicum</i>	0.68
<i>Ocimum tenuiflorum</i>	0.69
Gallic acid standard	0.70



Fig 1: TLC plate showing the presence of phenols

Conclusion

The results obtained in the present study suggest that the leaves of *Ocimum basilicum* and *Ocimum tenuiflorum* are good sources of healthy phytochemicals, essentially the phenolics. The presence of polyphenols in Sweet Basil and Holy Basil can be related to their antioxidant properties indicating that inclusion of these plants in human or ethnoveterinary formulations can contribute to potential health benefits in man, livestock and poultry.

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