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Research Article

Evaluation of Total Phenolic, Flavonoid Content and Antioxidant Activity of Essential Oil Extracted from Aromatic Plant Thai Basil Leaves (*Ocimum Basilicum Var. Thyrsiflorum*)

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Abstract

Introduction: Thai Basil (*Ocimum basilicum var.thyrsiflorum*) is a famous medicinal plant and an excellent source of vitamins, essential nutrients and antioxidant compounds with numerous health benefits, with action to prevent cancer, diabetes, and cardiovascular disease.

Aim: The aim of this study was to determine the proximate analysis, total flavonoid and phenolic contents including antioxidant activity of Thai basil leaves after oil extraction using Soxhelt method.

Methods: The proximate analysis was done using AOAC method. The total phenolic and flavonoid content were evaluated using colorimetric assay. Antioxidant activity was analysed using the in-vitro standard spectrophotometer method. Thai basil leaf extract was investigated for their in-vitro radical scavenging activities using 2, 2-diphenyl-1-picrylhydrazyl (DPPH).

Result: The proximate analysis in % showed that the Thai basil leaves contain the highest amount of moisture content (13.50%) and crude fat content (11.30%), moderate amount of ash (5.20%), crude fibre (9.80%), and crude protein (8.30%). The total phenolic and flavonoid content in the examined extract was found to be 79µg/ml and 51µg/ml on a dry matter basis. The leaf extract increases DPPH scavenging activity up to 81.735µg of concentration.

Keywords: Thai Basil Leaves; Proximate Analysis; Total Flavonoid; Total Phenolic; Antioxidant Activity

Introduction

Aromatic plants have been used worldwide since ancient time for their aroma, flavour and medicinal properties. Thai basil *(ocimum basilicum var. thyrsiflorum)* is one of the aromatic plants belonging to the mint family (Lamiaceae) and mainly distributed throughout temperate, tropical and subtropical regions of the world [1]. Thai basil originated from warm tropical countries such as Africa and Southeast Asia but is now broadly cultivated around the world [2]. Thai basil is a variety of basil native to Southeast Asia. It is widely used in Southeast Asian cuisine.

The Indian variety of Thai basil grows to an average of 30 to 40 centimetres (cm) in height and spreads about 30cm wide (Figure 1). The shape of the leaves is narrow, the colour of the leaves varies from bright-green colour to serrated edges. They also boast a fresh and spicy fragrance and sometimes have a slight purple tint. When the plant matures, spikes of lavender to dark-violet flowers grow at

the tops of the reddish-purple stems. Thai basil leaves, flowers, and stems are all edible and offer a bold flavour with spicy-sweet notes of anise and black licorice. Its dried leaves as well as its essential oil are used in the food industry as aromatic and flavouring ingredients. The essential oil of *ocimum basilicum var. thyrsiflorum* has gained wide importance, because of their many bioactivities. It is used in pharmaceutical agents because of their anticancer activity, anti-asthamatic, anti-stress, antimicrobial activity [3], anti-diabetic [4], and antioxidant activity [5].

The Thai basil leaves are used in the folk medicinal system; this herb has been used to cure sore throat, fever, common cold, cough, obesity, stress, insomnia, and even cancer disease [6-8]. The benefits are due to the presence of flavonoids, vitamins (vitamin A, vitamin C, vitamin E, vitamin K) fiber, and minerals (calcium, copper, Iron, Magnesium, manganese, phosphorus, potassium, selenium, sodium, zinc). Potassium is an important component of cell and body fluids, which helps control heart rate and blood pressure. Thai basil has notably high levels of vitamin A, which is beneficial for healthy eyes and skin, and vitamin K, which promotes bone health. Essential oils of Thai basil present compounds like eugenol, linalool, and limonene that possess antibacterial, anti-inflammatory, and antioxidant properties.

Antioxidants have potential therapeutic agents to prevent free radical generated damage in the human body. Antioxidants of natural origin, compared to the synthetic antioxidants present in the market, have attracted considerable attention by consumers and by researchers since there is concern of synthetic antioxidants consumption due to their instability and possible activity as carcinogens [14].

In recent years, consumption of herbal plants in the average diet has been highlighted for its contribution towards lowering the risk of lifestyle related diseases and the aging process because these are directly related to the active oxygen and lipid peroxidation [9]. Thai basil has a high level of antioxidants, which are the results of large groups of polyphenols and flavonoids such as quercetin. Natural antioxidants obtained from plants have certain advantages over synthetic ones, such as their easy and economical acquisition and slight or negligible side effects [14]. As a result, preserving products with natural preservatives has led to targeting herbs and spices as major sources of natural antioxidants [10].

The purpose of this research is evaluating total phenolic, total flavonoids and antioxidant activity of (*Ocimum basilicum var. thyrsiflorum)* extracted by petroleum ether (solvent) due to its accuracy in result.

Figure 1: Picture of Thai basil plant (Image by: Kyra_Sian, Candide, 2020).

Materials and Methods

Collection and preparation of plant material

Fresh leaves of Thai basil *(ocimum basilicum var. thyrsiflorum)* were collected from Babasaheb Bhimrao Ambedkar Central University, Lucknow (INDIA) during the Month of January 2022. Thai basil leaves were washed with tap water to remove dust and were allowed to sun dry for 72hours as it is a natural and easier method. 1 kg of the Thai basil leaves was sun dried and grinded using an electric blender to powder form and then stored in an airtight container to avoid it from an attack or certain environment. After that the dry powder was used for further extraction and further experiments.

Chemical and standards

All chemicals of analytical grade including: 2,2-diphenyl-2-picrylhydrazyl free radical (DPPH), 2,4,6 (tripyridyl)-1,3,5-triazine (TPTZ), hydrogen chloride (HCl), ferric chloride (FeCl3), ferrous sulphate 7-hydrate, acetate buffer (0.3M) at pH 3.6, gallic acid,

Folin-Ciocalteu phenol reagent, anhydrous sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), Quercetin, aluminium chloride ($AlCl_3$), and sodium hydroxide (NaOH) were obtained from Sigma-Aldrich India. Petroleum ether (solvent) was purchased from Gyan Scientific Traders India Pvt. Ltd. Lucknow.

Extraction preparation for total phenolic, flavonoid, and anti-oxidant properties

The extraction of plant material was performed at the lab of the Food and Nutrition Department, Babasaheb Bhimrao Ambedkar University, Lucknow. Extraction of dry Thai basil leaves has been done by using soxhlet method. In this method, the petroleum ether (solvent) is boiled at 40°C as long as 8-10hours. The sample of powdered leaves was finely put 50g into a thimble located in the middle part of the Soxhlet equipment. 250ml Petroleum ether solvent used for the extraction process is put in a round bottom. Once extraction is complete, volatile oil has separated from the solvent using a rotary evaporator.

The volatile oil sample obtained were thereafter kept in a sterilized dark bottle at ambient temperature (27 ± 3°C) until needed for use.

Proximate analysis

The dry Thai basil leaves powder was quantitatively analysed for chemical composition.

a) Moisture content, b) Ash content, c) Crude Fat, d) Crude Protein, e) Crude Fiber.

Moisture content

Ranganna method was used for estimation of moisture content [11]. Briefly, the moisture content of fresh Thai basil leaves can be determined by taking 5gram of sample in a previously sterilized Petri plate, weighing it in an electronic weighing balance, and placing them in a hot air oven for about 4-5hours at 80°C. Cool it in a desiccator for 15 minutes to absorb extra moisture and then the final reading of a Petri plate was taken. Moisture content can be calculated by using this following formula

Moisture content (%) = Weight of residue × 100 ÷ weight of dry leaves powder of Thai basil

Ash content

In this method, 4 gram of sample was weighed in a crucible by using an electronic weighing balance. After weighing, the crucible was placed in a muffle furnace (Model no. LSI ISO 9001:2008 Co.) for about 3 hours at a very high temperature i.e., 520°C. Afterward, cool the crucible in a desiccator to absorb extra moisture and weigh it [11].

The formula for determination of ash content

Ash content (%) = weight of ash × 100 ÷ weight of dry leaves powder of Thai Basil

Crude fat

Crude fat was estimated by Ether Extraction Method [16]. An extraction of 5 gram of dried sample was placed in the thimble of soxhlet extractor. Afterward, 250 ml petroleum ether (solvent) was added into a round bottom flask to boil at 40°C for 6 hours for the extraction process. Once extraction is completed then the collected sample was cool down. The collected sample containing solvent was evaporated via evaporator. Crude fat content can be calculated by using the following formula

% Crude fat = (weight of the beaker + Ether extract) - (weight of the beaker)/weight of sample × 100

Crude Protein

Protein contents were determined by Kjeldhal method of Bremner and Mulvaney [15]. % of crude protein was calculated using the following formula

Crude protein (%) = %N × 6.25

%N = (S-B) × N × 0.014 × D × 100/Weight of sample × V

Where, S: Sample Titration Reading; D: Dilution of Sample After Digestion; V: Volume Taken from Titration; N: Normality of HCl; B: Blank Titration Reading

Crude fiber

Under desired conditions, crude fiber was obtained from digestion of samples free from fat using 1.25% of sulphuric acid and sodium hydroxide solutions respectively. The crude fiber % was calculated by following formula

Crude fiber (%) = (loss weight on ignition)/weight of sample × 100

Antioxidant activity of leaves extract

Thai basil leaves has good antioxidant properties. It can be determined by it's a) phenolic content (TPC), b) flavonoid content (TFC), c) Scavenging activity (DPPH), and d) iron-reducing activity (FRAP).

Determination of total flavonoid content in extract

The total concentration of flavonoids in leaves extract was measured by the colorimetric assay technique [17]. Briefly, 1 mg of leaf extract was dissolved in methanol reagent and $NaNO_2$ solution (20%) of various concentrations (20,40,60,80,100 µg/ml). Thereafter, let it incubated at room temperature for 6 min. Thereafter, 0.3 ml of aluminium chloride (10% $AlCl_3$) solution was added and the reagents were further mixed and the reaction was allowed to stand for another 6 min. Finally, 2 ml of 1M sodium hydroxide (NaOH) solution was added to the mixture which was diluted up to 10ml of volume. and incubated for 10 min at room temperature. Absorbance was measured at 510 nm using a spectrophotometer (Model no. Evolution 201), and the measurements were calculated using a standard curve.

Determination of total phenolic content in extract

The total phenol in leaves extract was measured using the colorimetric assay process [17]. Folin-Ciocalteu reagent used in this method and as the stock solution gallic acid was used. Briefly, preparation of gallic acid (1 mg/ml) was made by combining 10mg of gallic acid (20,40,60,80,100 µg/ml) in methanol and leaves extract. Add 1ml of sodium carbonate (Na_2CO_3) to 1ml of the folin reagent at each concentration, using distilled water to increase the volume up to 10 millilitres. The mixture could be kept at room temperature for 30 min and the optical density was measured at 765 nm using a UV Visible spectrophotometer (Model no. Evolution 201).

Determination of antioxidant activity (DPPH)

The antioxidant activity of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) is calculated via spectrophotometer [12] with small modifications [18]. In methanol, the color of DPPH is dark blue. In its reduced form, the antioxidant compound changes color from purple to yellow, allowing DPPH to gain electrons. DPPH shows strong absorption at 517 nm, determined by 2, 2-diphenyl-22-pyridyl hydroxylase (DPPH). Briefly, 0.1 ml DPPH solution was mixed with 1ml of extract leaves prepared in various concentrations (20,40,60,80,100 µg/ml). A control sample of 1 ml of methanol was prepared and incubated in the darkroom for 30 minutes at ambient temperature. After incubation, the absorbance of the sample was read at 517 nm using a UV Visible spectrophotometer methanol used as a blank. Reduction in the absorbance value, shows high activity in scavenging free radicals [19].

It was measured as a percentage of DPPH scavenging activity by using the following formula given below:

$$\text{DPPH scavenging activity} = (A_{control} - A_{control})/A_{control} \times 100$$

Note: The test tube was covered with brown paper as DPPH is very sensitive to light.

Ferric reducing antioxidant power assay

The reducing and antioxidant capacity of iron is determined by [20] and with slight modifications [21]. The following three reagents were used to prepare for FRAP analysis. Briefly, 10mM of 2,4,6 (tripyridyl)-1, 3, 5-triazine (TPTZ) dissolved in 40 mM HCl, 20mM $FeCl_3$ in H2O and 0.3 mM acetate buffer (pH 3.6), all three-solution mixed in the ratio 1:1:10. The FRAP reagent contained 5ml TPTZ solution, 5ml FeCl3 solution and 40 ml acetate buffer. It was freshly prepared and warmed to 37^0 C. Then 2ml FRAP reagent was added to 1ml of plant extract at different concentrations of (10, 20, 30, 40, 50 µg). The reaction mixture was then incubated at 37^0 C for 30 min and then absorbance was recorded at 595 nm. An intense blue colour complex was formed when Ferric tripyridyl-triazine (Fe^{3+} TPTZ) complex was reduced to the ferrous (Fe^{2+}) form. Hence, to estimate the concentration of ferrous liberated due to extract activity a calibration curve of feSo4 in the range 5-25 µM concentration was plotted. The concentration of ferrous in the reaction mixture of extract was estimated by using their optical density and equation generated on the standard curve. The result for the FRAP activity at variable concentration is represented as µM of ferrous form.

Results and Discussion

Proximate analysis result

The result of proximate composition (Table 1) shows that the highest moisture content of the leaf sample was 13.50%, the fat

content was 11.30% which showed that the plant leaf contained good oil quantity. The crude fiber (9.80%), ash (5.20%) and the protein content found to be 8.30% in *ocimum basilicum var. thyrsiflorum.*

Parameters	(%) Proximate composition
Moisture (per 5gm)	13.50%
Ash content (per 4gm)	5.20%
Crude fat (per 5gm)	11.30%
Crude fiber	9.80%
Crude protein	8.30%

Table 1: Result of the proximate analysis of the osmium basilicum var. thyrsiflorum.

Total Flavonoid Content

In this study, total flavonoid content of *ocimum basilicum var. thyrsiflorum* was determined as shown in table 2. The equation from the Quercetin standard curve was used to determine the concentration of Total Flavonoids content in sample leaf extract of Thai basil. The absorbance value of extract at 510 nm was 0.167, so putting the same at the place of y in the equation; the concentration of TFC for extract was 51µg/ml as shown in figure 2.

Total phenolic content

The total content of phenol in the leaves extract is expressed in Gallic acid equivalent. The total phenolic content can be determined from the standard curve equation. The equation from Gallic acid curve was used to determine the concentration of Total phenol content in sample leaf extract of Thai basil. The absorbance value of extract at 765 nm was 0.746, as given in table 3; so at the same at the place of y in the equation, the concentration of TPC for extract was 79µg/ml, given in figure 3. The variation between TPC and TFC of different plant materials can be different chemical composition, soil, condition, and maturity of a plant [22].

Concentration (μg/ml)	Abs at 510 nm
0	0
20	0.088
40	0.153
60	0.211
80	0.271
100	0.329

Table 2: Showing the absorbance obtained for Quercetin standard at 510 nm.

Concentration (μg/ml)	Abs at 765 nm
0	0
20	0.255
40	0.452
60	0.629
80	0.821
100	0.997

Table 3: Showing the absorbance obtained for Gallic acid standard at 764 nm.

Figure 2: Graph showing the standard curve of Quercetin.

Figure 3: Graph showing the standard curve of Gallic acid.

Antioxidant radical scavenging activity

DPPH is the most suitable way to determine the antioxidant property of a sample [13]. Because DPPH free radicals are scavenged by antioxidant compounds, the colour of the sample changes from purple to yellow (Nirmala). Figure 4 shows the graph between concentration (µg) and antioxidant activity (%) of extract. By using a spectrophotometer, the optical density of a sample and the optical density of the control can be calculated to determine DPPH behaviour in a sample. According to [23], if DPPH value is below 50 µg/ml it has a very strong antioxidant property, if it lies between 50-100 µg/ml has strong antioxidant property and if it is above 150 µg/ml it has weak antioxidant property. The antioxidant activity of Thai basil leaves extracted at different concentrations (20, 40, 60, 80, 100 and 120µg) was evaluated in table 4 and the results obtained were illustrated in figure 3. According to these results, leaves extract concentration increases up to 81.735µg. Afterward, the activity of antioxidants was constant. The equation generated on the above curve was used to determine the IC50 value, where y was replaced with 50 and value of x was calculated which indicated the concentration of extract at which 50% DPPH radicals were scavenge. At the concentration of 81.735 µg/ml, the antioxidant activity of leaves extract was maximum, thereafter it showed constant activity.

Concentration	Abs at 517 nm	Abs. Control	% Scavenging	IC50 value
20	0.168	0.211	20.379	81.735µg
40	0.154		27.014	
60	0.128		39.336	
80	0.111		47.393	
100	0.085		59.715	

Table 4: Showing the absorbance value obtained for Thai basil leaf oil extract for DPPH radical scavenging activity.

Figure 4: DPPH activity of Thai basil oil extract.

FRAP analysis

When increase in extract concentration the Ferric reducing capacity was also increased as represented by the concentration of Ferrous sulphate formed (Table 5) under the assay condition at different concentrations of extract (Table 6). Figure 5 shows the standard curve for ferrous sulphate.

Concentration of $FeSO_4$ (µM)	OD at 595 nm
0	0
5	0.022
10	0.045
15	0.063
20	0.082
25	0.101

Table 5: Showing the absorbance value obtained for $FeSO_4$ standard at 595nm**.**

Figure 5: Graph showing the standard curve of ferrous sulphate.

Concentration of extract (µg)	OD at 595 nm	μM of $FeSO_4$
10	0.107	26.25
20	0.116	28.5
30	0.123	30.25
40	0.128	31.50
50	0.133	32.75

Table 6: Showing the amount of $FeSO_4$ liberated at different extract concentration under FRAP assay.

Conclusion and Recommendations

Thai basil leaves has a great role ranging from food to cosmetics to pharmaceuticals. It has potential health benefits as Thai basil

contains antioxidants that act as a natural anti-inflammatory which fights many diseases. It protects against bacterial infection as well as against virus. It promotes heart health and also has cancer fighting properties. It regulates normal liver functioning and helpful in combating stress.

The conclusion of this study is that the Thai basil leaves (*ocimum basilicum var. thyrsiflorum)* contain good nutritional property. The obtained value from proximate analysis shows that it has good moisture content and fibre content. The unique health benefits of Thai basil are due to its very high antioxidant content. For example, phytochemicals such as phenolic and flavonoids found in Thai basil contribute to various disease prevention.

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Conflict of Interest

The authors declare that they have no known conflict of interests or personal relationships that may affect the work reported in this paper.

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