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Screening of phytochemical, antioxidant and anti-inflammatory properties of Thymus vulgaris

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ABSTRACT

Thymus vulgaris, commonly known as thyme, is a culinary herb with a long history of traditional medicinal use. The present study involves the extraction of phytochemicals using various solvents of Thymus vulgaris extracts. The extracts were subjected to qualitative phytochemical analysis and quantification of important phytochemicals such as flavonoids, alkaloids and tannins was also done. The antioxidant capacity and anti-inflammatory potential of Thymus vulgaris methanolic extract was evaluated through DPPH scavenging assay and proteinase inhibition assays. This work provides valuable insights into the phytochemical profile of Thymus vulgaris and its potential applications in combating oxidative stress and inflammation-related disorders. This research may contribute to the development of natural remedies and pharmaceuticals with antioxidant and anti-inflammatory properties derived from Thymus vulgaris.

Key words: Thymus vulgaris, Flavonoids, phytochemical, antioxidants and anti-inflammatory.

1. INTRODUCTION

Thymus vulgaris is a flowering plant of the family Lamiaceae commonly known as thyme, native to Southern Europe, and has a worldwide distribution (Hosseinzadeh et al., 2015). The plant is indigenous to the Mediterranean and neighboring countries, Northern Africa, and parts of Asia. In Africa, the plant has been cultivated in Egypt, Morocco, Algeria, Tunisia, Libya (Stahl-Biskup and Sáez, 2002), Cameroon (Nkouaya Mbanjo et al., 2007), Nigeria (Kayode and Ogunleye, 2008), and South Africa (Schmitz, 2015). People have used thyme for many centuries as a flavoring agent, culinary herb, and herbal medicine (Stahl-Biskup and Venskutonis, 2012). The plant is useful as infusion to treat cough, diabetes, cold and chest infections; and in a syrup form for digestive upset.

It is also soothing for sore throat, as thyme has antiseptic, antibiotic and antifungal properties (Ekoh et al., 2014). Due to its use as incense, balsamic odour, or because it belonged to the class of herbs with sweet scents, the Greek term "thyme" means "to fumigate". In many nations, it is typically produced for commercial purposes in order to produce dried leaves, plant extracts, Oleoresins and plant oils. Due to its wide aromaticity T. vulgaris. is utilised commercially as a flavouring agent in the food industry. Along with its use for flowering and decorative purposes, it is also used for preserving beef, fowl and fish. T. vulgaris. is widely used in the cosmetic and perfume industries for its distinctive scent.

Thyme is applied to the skin. Thyme is said to treat bites and stings, neuralgia, rheumatic aches, and pains when applied topically (Reddy et al., 2014). According to Ekoh et al. (2014), the essential oil can be applied topically to relieve rheumatic or joint discomfort as well as athlete's foot (Tinea pedis, V. Kuete, 2017). The essential oil is responsible for the typical spicy aroma of thyme (Stahl-Biskup and Venskutonis,2012). The predominant compound among the essential oil components was identified as thymol (compound 9; 51.34%) while the amount of all other components was less than 19% (Reddy et al., 2014). Dried Thymus vulgaris plant contains 1–2.5% of essential oil (Stahl-Biskup and Venskutonis, 2012).

Thyme oil (TO) is derived from the flowers of various plants belonging to the genus Thymus. It has been used as a therapeutic agent since ancient times. The thyme essential oil may be recommended for large-scale application as a plant-based preservative for stored food items because of its strong antifungal as well as antiaflatoxigenic efficacy. This oil showed highest antifungal efficacy. The thyme oil absolutely inhibited the mycelial growth of Aspergillus flavus and exhibited a broad fungitoxic spectrum against eight different food-contaminating fungi. The chemical structures of the major terpenoids of the essential oil are quoted (Reddy et al., 2014). Most of the volatiles detected in thyme oil belong to the monoterpene group with compound 9, a phenolic monoterpene, as the main representative (30–55%) (Reddy et al., 2014).

Aside from the essential oil, the tannins, mainly represented by rosmarinic acid (Fig. 27.2), contribute to the commercial use of the herb (Kivilompolo and Hyoetylaeinen, 2007). The contents of rosmarinic acid reported in the literature vary between 0.15 and 4 %. Also the 3'-O-(8"-Z-caffeoyl) -rosmarinic acid has been isolated from the leaves (Dapkevicius et al., 2002). Free phenolic acids are mainly represented by caffeic acid, gentisic acid, p-cumaric acid, syringic acid, ferulic acid and p-hydroxybenzoic acid (Proestos et al., 2005; Kivilompolo and Hyoetylaeinen, 2007). The phytochemical constituents of thyme include phenolics, terpenoids, and mostly thymol, eugenol, and saponins (Ekoh et al., 2014). Thyme essential oil showed a high content of oxygenated monoterpenes (56.53%) and low contents of monoterpene hydrocarbons (28.69%), sesquiterpene hydrocarbons (5.04%), and oxygenated sesquiterpenes (1.84%) (Reddy et al., 2001). Thyme also possesses carminative and antioxidative effects. Fachini-Queiroz et al. (2012) showed that the constituents, thymol and carvacrol, present effects on the inflammatory response; the anti-

inflammatory properties of TEO are partially involved in the hepatoprotective effect of the essential oil (Grespan et al., 2014).

MATERIALS AND METHODS

Collection of Plant material

The leaves of Thymus vulgaris were collected from a garden, Bangalore. The collected plant leaves were washed with tap water; shade dried at room temperature. In general the plant material should be dried at temperature below 30^0C to avoid the decomposition of thermolabile compounds. So sun drying can be very effective but drawback is sometimes water molecules are absorbed by the sample and hence microbial growth can affect the phytochemical study. The leaves were dried in the sun light thus chemical decomposition cannot take place.

Grinding of Dried sample

Small amount of plant material can be milled using grinder or blender. However, if the sample is in large quantities, using a spice mill to ground it into powder is easier. Grinding increases surface area, which boosts the effectiveness of extraction. Additionally, it reduces the quantity of solvent needed for the extraction. The dried materials were mechanically ground into a coarse powder (Blender), and the powdered samples were stored in sterile, tightly-sealed glass containers while extraction was taking place. To prevent contamination from any leftover previously ground material or other foreign matter placed on the grinder, the grinder was carefully cleaned before and after the sample was ground.

Sample Preparation

Shade-dried plant leaves chopped into small pieces by electronic blender and ground into powdered form. The powdered plant material was subjected to sequential solvent extraction by soxhlet extraction method. 50 gm of dried plant powder was packed in sterile filter paper and it was loaded in a clean and dried thimble of soxhlet apparatus carefully. It was then fitted with a pre -dried round bottom flask having a capacity of 500 ml. Soxhlet apparatus was then set up. The flask was constantly heated using an electric heating mantle with controlled temperature. The process was continued until the colourless solvent appeared from plant material in soxhlet apparatus. The plant extracts were collected in a round bottom flask. Concentrated extracts were subjected to various chemical tests in order to detect various phyto-constituents.

Qualitative Phytochemical tests

Preliminary phytochemical analysis was carried out for all the extracts as per standard methods described by Brain and Turner 1975 and Evans 1996. Methanolic extracts of T.vulgaris obtained by the above method were subjected to qualitative analysis for identification of the

presence of Phenolic groups, Glycosides, Alkaloids, Flavonoids, Tannins, Terpenoids, Saponins

Quantitative Estimation of Alkaloids

To 1 ml extract, 2 ml of phosphate buffer (pH 4.7) and 0.5ml of bromocresol green solution was added. Mixed it well and added 1.5ml of chloroform. Blank was prepared by adding methanol instead of extract. The absorbance of the complex in chloroform was measured at 470 nm against blank. Atropine wass used as standard. The alkaloid concentration was determined by comparing with standard.

Quantitative Estimation of Flavanoids

Total flavonoids content was determined by the Aluminium chloride method using catechin as a standard. 100µl of test samples were added to the test tube. After 5 min 150µl of aqueous sodium nitrate and 150µl of 0.5M Aluminum chloride was added. After 6 min incubation at room temperature, 1ml of 1M sodium hydroxide was added to the reaction mixture. The absorbance of the reaction mixture was measured at 490 nm against a blank spectrophotometrically.

Quantification of Anti-inflammatory activity

The test samples were treated according to the procedure to check for its anti-inflammatory activity. The action of proteinase inhibition of the plant sample was measured using trypsin buffer with casein protein. Here methanol was used as control and the sample OD at 280nm was compared to the control. The anti-inflammatory activity was calculated using the following formula.

Percentage inhibition = (Abs Control - Abs of Sample) * 100/ (Abs Control)

Quantification of Antioxidant activity

The test samples were treated according to the procedure and the OD was measured at 695 nm. The antioxidant activity was calculated using the ascorbic acid as standard. The absorbance of ascorbic acid at 1mg/ml concentration of ascorbic acid was found to be 0.24 which was used in the below formula to get the antioxidant activity of test sample

DPPH radical scavenging activity

The antioxidant activity is expressed as the number of equivalents of ascorbic acid. The assay is based on the reduction of Mo(V1) to Mo (V)by the sample analyte and the subsequent formation of a green phosphate Mo (V) complex at acidic pH with the maximal absorption at 695nm (5 ug/ml) as standard as at this concentration, absorbance becomes almost constant (Sony et al., 2014). The basic principle is the scavenging ability of the leaf extract on the ammonium phosphomolybdenum reagent. 1ml of the extract was mixed with 4.5 ml phosphomolybdate reagent and incubated at 95° C temp for 90 minutes, optical density was

measured at 695nm. The samples were prepared in triplicate and the average of the three-readings obtained was taken as the final absorbance. Total antioxidant capacity was calculated by the formula.

Total antioxidant capacity (TAC)% = (As-Ac) / (Aaa -Ac)

Where, A_c =Absorbance of control

A_s = Absorbance of sample

A_{aa} = Ascorbic acid absorbance

Total antioxidant activity was also determined in terms of ascorbic acid in mg/ml

Anti-inflammatory assay

Anti-proteinase action / Proteinase inhibitory action

The reaction mixtures (2.0ml) in the test tubes contained 1ml of 0.06 mg trypsin + 25mM tris-HCL buffer (pH 7.4) and 1 ml of the sample extract (100mg/ml). The mixture was incubated at 37^0C for 5 minutes. Then 1.0 ml of 0.8% (w\v) casein was added. The mixtures were incubated at 37^0C for 20 minutes. After incubation 2 ml of 70% (v\v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged at 8000 rpm for 5 minutes and read the absorbance at 280 nm against the buffer as blank.

Statistical Analysis

There were three separate tests done for each experiment. The mean and standard deviation (Mean±SD) is used to describe the spread of the data. Microsoft Excel 2007 (Roselle, Illinois, United States) was used for statistical analysis.

RESULTS

Qualitative Phytochemical Analysis

The present study was carried out to screen the phytochemicals from methanolic extract of leaf part of Thymus vulgaris which revealed the rich variety of chemical constituents. Alkaloids, flavonoids, phenols tannins, quinones, and saponines were present in the extract. Aminoacids, Cardiac glycosides, Catrbohydrates and terpenoids were absent in methanolic extract. Phytochemicals are the core of phytomedicines; their therapeutic efficiency directly correlates with the presence of various phytochemicals. In this study, we have identified and quantified the major phytochemicals present in the leaves of Thymus vulgaris. Therefore, phytochemicals play a major role in the prevention of diseases and disorders thus, used for medicinal purposes (The World Health Organization). The medicinal property of Thymus vulgaris may be due to the presence of the phytochemicals. Thus the phytochemical screening tests may be useful in the

detection of the bioactive principles and subsequently may lead to the drug discovery.

Sl.No	Phytochemicals	Result
1.	Alkaloids	+
2.	Carbohydrates	-
3.	Cardiac Glycosides	-
4.	Flavonoids	+
5.	Phenols	+
6.	Amino Acids	-
7.	Saponins	+
8.	Terpenoids	-
9.	Quinones	+
10.	Tannins	+

Table 1: Qualitative phytochemical analysis of methanolic extract of Thymus vulgaris.

Quantitative Test for Flavanoids:

Concentration (mg/ml)	OD 1	OD 2	OD 3	Mean	SD
0.00	0.000	0.000	0.000	0.000	0.000
0.02	0.015	0.019	0.020	0.018	0.002
0.04	0.037	0.034	0.033	0.035	0.002
0.06	0.062	0.057	0.058	0.059	0.002
0.08	0.079	0.074	0.073	0.075	0.003
0.10	0.102	0.095	0.094	0.097	0.004

Table 2: Absorbance of Standard Catechin

Table 3: Estimation of Total Flavonoid Content.

	OD 1	OD2	OD 3	Mean	S.D	Concentration mg/ml
Sample	0.27	0.43	0.21	0.30	0.11	0.301

Methanolic Extract of Thymus vulgaris contains 0.301 mg/ml of flavonoid content compared with standard.

Quantitative test for phenols

Table 4 : Absorbance of standard gallilic acid.

Concentration (mg/ml)	OD	OD 1	OD 2	OD 3	Mean	SD
0	0	0.00	0.00	0.00	0.00	0.00
0.2	0.1	0.12	0.08	0.11	0.10	0.02
0.4	0.23	0.21	0.24	0.24	0.23	0.02
0.6	0.35	0.38	0.33	0.35	0.35	0.03
0.8	0.49	0.46	0.51	0.49	0.49	0.03
1	0.6	0.58	0.61	0.60	0.60	0.02

Table 5: Estimation of Total Phenol Content

	OD 1	OD 2	OD 3	Mean	S.D	Concentration in mg/ml
Sample	0.16	0.18	0.12	0.46	0.16	0.758

Methanolic extract of Thymus vulgaris contains 0.758 mg/ml of phenol content compared with standard.

Table 6: Quantitative Test for Tannins.

Concentration (mg/ml)	OD	OD 1	OD 2	OD 3	Mean	SD
0	0	0.00	0.00	0.00	0.00	0.00
0.2	0.1	0.12	0.08	0.11	0.10	0.02
0.4	0.23	0.21	0.24	0.24	0.23	0.02
0.6	0.35	0.38	0.33	0.35	0.35	0.03
0.8	0.49	0.46	0.51	0.49	0.49	0.03
1	0.6	0.58	0.61	0.60	0.60	0.02

Table 7: Estimation of Tannin

	OD 1	OD 2	OD 3	Mean	S.D	Concentration mg/ml
Sample	0.20	0.25	0.26	0.71	0.03	0.728

Methanolic Extract of Thymus vulgaris contains 0.728 mg/ml of tannin content compared with

standard.

Results of antioxidant activity assay

Calculation:

Total antioxidant capacity (TAC)% = $(A_s-A_c) / (A_{aa} -A_c) * 100$

= (0.12-0.0) / (0.87-0.0) * 100

= 13.79%

Where, A_c =Absorbance of control, A_s = Absorbance of sample, A_{aa} = Ascorbic acid absorbance at 1mg/mL

The Total antioxidant capacity (TAC)% of the plant extract was found to be 13.79%.

Results of anti-inflammatory activity assay

Calculation:

Percentage inhibition = (Abs Control - Abs of Sample) * 100/ (Abs Control)

= (2.067-1.694) *100/2.067

= 18.04%

Where, Ab_c Control - Absorbance of Control, Ab_s Sample - Absorbance of sample

The Total anti-inflammatory capacity (TAC)% of the plant extract was found to be 18.04 %.

DISCUSSION AND CONCLUSION

In recent days, significant advances in experimental methodology and molecular biology have enabled researches to investigate the potential use of phytochemicals to treat or manage a plethora of chronic diseases including cancer, diabetes, inflammatory diseases and cardio vascular abnormalities. Many of today's drugs are derived from land sources. Plants have been used in medicine throughout the world and still continue to occupy and important place in traditional as well as modern system of medicine. In today's world the percentage of people using chemical and drugs are increasing with their side effects. "The boon given to our earth is the herbs", which needs to be utilized in sustainable manner.

Nature is full of medicinal plants and these are rich source of therapeutic agents for the prevention of various diseases. Among such medicinal plants Thymus vulgaris is one such plant which is used to treat several diseases, extensively used in Ayurveda, and homeopathic medicine. Thyme has several phytochemicals which have been isolated from different parts of thyme those phytochemicals has several pharmacological properties.

In the present study Thymus vulgaris plant was subjected to soxhlet extraction with methanol and various qualitative analysis were performed which suggested the presence of phytochemicals like flavonoids, phenol, saponins, tannins, quinone and alkaloids.Thus, these

phytochemicals were quantitated using the respective standards. The quantitative analysis of these phytochemicals showed amount of flavonoids, phenol, saponins, tannins, quinone and alkaloids to be 0,301, 0.758, 0.410, 0.728, 0.274 mg/mL respectively. The antioxidant activity of thymus vulgaris was found to be 13.79% while its anti-inflammatory activity showed 18.06% proteinase inhibition. All these phytochemicals impart thymus vulgaris its unique qualities and its potential to cure many diseases. The antioxidant potential of the combined extracts is not only controlled by the concentration of phenolic compounds, but it is also dependent on the structure and the synergistic interactions between phenolic compounds against the oxidation process. As observed, all studied extracts had anti-inflammatory activity and antioxidant capacity. Among the extracts, the highest reducing power, total antioxidant capacity, and anti-inflammatory were obtained from the thyme mixture, which can be attributed to the increased phenolic and flavonoid contents. These results represent a basis for further research on the potential use of the combination of thyme as natural antioxidants and anti-inflammatory agents, both in the food and pharmaceutical fields.

Recent researches have shown that active ingredients such as triterpenoids and flavenoids promote wound-healing activity against methicillin resistant, antimicrobial properties, which seem to be responsible for wound contraction and an increased rate of epithelialization.

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Conflict of Interest

The authors state that they have no conflict of interests.

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