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Research Article

QUANTIFICATION OF TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT OF EXTRACTS OF *TAGETES ERECTA* FLOWERS

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ABSTRACT

Objective: *Tagetes erecta (T. erecta)*, marigold, has various ethnomedicinal uses. It has a wide variety of bioactive components such as polyphenols and flavonoids which show different bioactivities. The aim of the present study is to quantitatively estimate total phenolic content (TPC) and total flavonoid content (TFC) of different extracts of *T. erecta* flowers.

Methods: Extraction was done by maceration process, sequentially from non-polar to polar. Chloroform, ethyl acetate, and methanol extracts of *T. erecta* flowers were subjected to preliminary phytochemical screening. The extracts were analyzed for TPC and TFC using gallic acid and rutin as standard, respectively.

Result: Phytochemical screening of different extracts showed the presence of carbohydrates, flavonoids, phenolics, fats, and oils. TPC and TFC in extracts of *T. erecta* varies with solvents. The study revealed that methanolic extract possesses the highest phenolic content, 49.76±0.29 mg gallic acid equivalents/g extract, and also maximum flavonoid content, 13.43±0.43 mg RE/g extract, among the three extracts.

Conclusion: Higher value of phenolics and flavonoid indicates higher antioxidant activity. The present study revealed that methanolic extract has the highest phenolic and flavonoid content. This indicates that the flowers may possess a good antioxidant property and further research could be carried out.

Keywords: Polyphenols, Flavonoids, *Tagetes erecta*, Total phenolic content, Total flavonoid content, Antioxidant.

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INTRODUCTION

Almost all herbs have medicinal properties and have useful effect on human health, for example, antioxidant activity, anti-inflammatory, antimicrobial, antimutagenic, and anticarcinogenic effect. The uses of herbs and medicinal plants in the treatment of various ailments have been known from ages. A variety of compounds have been isolated from herbal medicines which have been reported to effective against neurodegenerative and cardiovascular diseases. These compounds belong to catechols, stilbenoids, flavonoids, phenylpropanoids and lignans, phenylethanoids, glycosides, and terpenes [1]. At present, herbal and ayurvedic drug treatment is not only used in India but also has global commercial market [2].

Many bioactive components are considered to be available in medicinal plants. Flavonoids represent the largest category of plant phenolics and play a major role in defending against degenerative disorders. They have an important role in growth, development, and defense against injury. They also provide color to fruits, flower, and leaves [3]. Phenolics include phenolic acid, stilbene, tannins, lignans, and lignin. Their antioxidant activity is because of their redox properties due to which they act as reducing agent, hydrogen donator, and singlet oxygen quencher. Phenolic acid possesses carboxylic acid functional group. They work against oxidative damage. Alkaloids are alkaline or basic in nature and containing heterocyclic nitrogen atom ring. They are divided according to the heterocyclic ring present. They are pyridine alkaloids, pyrrolidine alkaloids, pyrrolidine pyridine alkaloids, pyridine, piperidine alkaloids, quinoline alkaloids, and isoquinoline alkaloids [4].

Flavonoids, a group of plant phenolics, are the most important phytochemical constituent present in plants. They are responsible as the chief coloring agent in flowering plants. They are an essential part of animals and human diet. They have been consumed by humans since ages. They have a broad range of biological properties which help in the reduction of various degenerative diseases, cardiovascular diseases, cancers, and other age-related diseases [5]. They show various bioactivities, most important being antioxidant, that is, ability to reduce free radical formation and to scavenge free radicals. Due to this, they have been a subject of study in the past years, and their structure-activity relationship has also been established [6].

Tagetes erecta has commercial and ethnomedicinal use. The plant belongs to family Asteraceae or Compositae. It is commonly called as marigold, widely used as herbal medicine. Every part of this plant has its use in folk medicine to cure various diseases. The leaves have been reported to be used

against piles, kidney troubles, muscular pain, ulcers, wounds, and earache. The pounded leaves are effective as an external application to boils and carbuncles [7].

The flower of *T. erecta* has a wide range of medicinal use against fevers, epileptic fits, astringent, carminative, stomachic, scabies, liver complaints, and diseases of the eyes. Flower juice is given as a remedy for bleeding piles and also used to purify blood, also used against rheumatism, cold, and bronchitis [8]. The plant shows antioxidant, antimycotic, antibacterial, antimicrobial, larvicidal, insecticidal, mosquitocidal, and nematicidal activity [9].

Flowers of *T. erecta* are an important source of carotenoids which has wide application in food industry [10]. Lutein is the major pigment present in marigold flowers [11]. It is a carotenoid with antioxidant property [12]. The antioxidant activity of phenol is found to be much greater than beta-carotene and lycopene [13]. Flavonoids extracted from marigold flowers were patulitrin and patuletin. They were isolated

and their structures established using nuclear magnetic resonance and high-performance liquid chromatography-mass spectrometry. These were also investigated for their dyeing process [14].

Preliminary studies carried on *T. erecta* have proved that the flowers are highly rich in phenolic compounds, phenolics, terpenes, etc [15]. In the present investigation, quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) of different extracts of flowers of *T. erecta* was done.

METHODS

Plant material

The plant was identified and authenticated by Dr. Zia ul Hasan, Head, Department of Botany, Saifia College, Bhopal. The voucher specimen number is 518/Bot/Saifia/2015. The flowers of *T. erecta* were dried under the sun for 7-10 days. After that, they were dried in oven at a temperature <60°C to remove the moisture content. The plant material is then grinded in a mechanical grinder to make a fine powder.

Preparation of extract

The material is extracted by maceration process, sequentially from non-polar to polar solvents with petroleum ether, chloroform, ethyl acetate, and methanol, respectively. The fractions were evaporated to dryness and stored for further investigation.

Reagents and chemicals

Gallic acid, methanol, Folin-Ciocalteu Reagent (1:10 in deionized water), sodium carbonate solution (7.5% w/v), rutin/quercetin, methanol, 5% $NaNO_2$, 10% $AlCl_3$, and 4% NaOH.

Preliminary phytochemical screening of the different plant extracts

Qualitative phytochemical testing of extracts was done to study the presence or absence of various phytochemical constituents using standard tests [16]. Phytochemical screening of different extracts showed the presence of carbohydrates, flavonoids, phenolics, fats and oils, saponins, etc.

Quantitative estimation of total phenolic constituents

TPC of all the extracts was determined by Folin-Ciocalteu method [17,18]. Gallic acid was used as standard phenolic compound. Different concentrations of gallic acid (10-100 µg/ml) were prepared in methanol. Test sample of each extract was prepared in methanol (100 µg/ml) or solvent of near about same polarity. A volume of 0.5 ml of different concentrations of gallic acid/test sample was added with 2 ml of Folin-Ciocalteu reagent followed by 4 ml sodium carbonate solution. The reaction mixture after that was incubated at room temperature and allowed to stand for 30 minutes with intermittent shaking. The absorbance was taken at 765 nm using methanol as blank. Standard curve of different concentrations of gallic acid was prepared to find the line of regression. The TPC was obtained from calibration curve of gallic acid and expressed as mg/g or µg/mg gallic acid equivalent (GAE).

Quantitative estimation of total flavonoids

Estimation of TPC was determined using colorimetric assay [19]. Different concentrations of rutin (10-100 µg/ml) were prepared in methanol. Rutin is used as standard for the preparation of calibration curve. Test sample of each extract was prepared in methanol (100 µg/ml) or solvent of near about same polarity. A volume of 0.5 ml of the diluted sample solution was mixed with 2 ml of distilled water followed by 0.15 ml $NaNO_2$ solution. After 6 minutes, 0.15 ml $AlCl_3$ solution was added and allowed to stand for 6 minutes. After that, 2 ml NaOH solution added to the reaction mixture and allowed to stand for 15 minutes. The absorbance was measured 510 nm using water as blank by ultraviolet spectrophotometer. Absorbance of test samples was measured by line of regression of standard curve of rutin. TPC is expressed as Rutin equivalent (RE), mg RE/g extract.

Statistical analysis

All the experimental data were replicated three times, and the results were expressed as mean±standard deviation of three replicates.

RESULTS

Phytochemical screening

Phytochemical screening revealed the presence of bioactive components such as alkaloids, carbohydrate, flavonoids, glycosides, phenolics, tannins, proteins, saponins, and polysaccharides in the extract (Table 1).

TPC

The TPCs of *T. erecta* extracts were calculated with a regression equation based on a standard curve using gallic acid. The methanolic extract had the highest phenolic content, 49.764±0.29 mg GAE/g extract. The lowest value obtained for chloroform extract, 15.450±0.44 mg GAE/g extract (Table 2).

TFC

The TFCs of *T. erecta* extracts were calculated with a regression equation based on a standard curve using rutin, (y=0.0023x+0.0531, R^2=0.9915). The methanolic extract (13.434±0.43 mg RTE/g extract) and chloroform extract (7.057±0.66 mg RE/g extract) showed the highest and lowest flavonoid content, respectively (Table 3).

DISCUSSION

Phenolics are the most important secondary metabolites present in plants [20]. They contribute to the antioxidant activity of plants due to their redox properties. They act as hydrogen donators, reducing agents, and oxygen scavengers [21]. The present study showed that *T. erecta* flower extracts have a good phenolic content, hence can act as a potent natural antioxidant. Flavonoids are another most significant bioactive

Table 1: Phytochemical constituents present in different extracts of flowers of *T. erecta*

Phytochemical constituent	Chloroform	Ethyl acetate	Methanol
Carbohydrates	+	+	+
Proteins and amino acids	-	-	-
Flavonoids	+	+	+
Alkaloids	-	-	-
Triterpenoids	+	+	-
Tannin and phenolics	+	+	+
Fats and oils	+	+	+
Steroids	-	-	-
Saponins	-	-	+
Glycosides	-	-	-

+: Indicates presence and, -: Indicates absence of phytochemical constituent, *T. erecta: Tagetes erecta*

Table 2: Total phenolic content of extracts of flowers of *T. erecta*

Extracts	Total phenolic content (mg GAE/g extract)
Chloroform	15.45±0.44
Ethyl acetate	44.47±0.58
Methanol	49.76±0.29

x represents quantity/concentration of phenols which is obtained from the equation: y=0.0034×+0.0028, where y is absorbance of samples and R^2=0.9815. Results are expressed in mean±SD (n=3), SD: Standard deviation, *T. erecta: Tagetes erecta,* GAE: Gallic acid equivalents

Table 3: Total flavonoid content of extracts flowers of *T. erecta*

Extracts	Total flavonoid content (mg RE/g extract)
Chloroform	7.05±0.66
Ethyl acetate	13.28±0.66
Methanol	13.43±0.43

x represents quantity/concentration of flavonoids which is obtained from the equation: y=0.0023×+0.0531, where y=absorbance of samples and R=0.9915. Results are expressed in mean±SD (n=3), SD: Standard deviation, *T. erecta: Tagetes erecta*

component [22]. The best property of flavonoids is their capacity to act as antioxidants. They show protective effect against various diseases. Flavones and catechins show a most significant antioxidant effect against reactive oxygen species [23]. The extracts show considerable flavonoid content, highest amount shown by methanolic extract.

CONCLUSION

The results showed that the methanolic extract had the highest phenolic and flavonoid content. The phenolics and flavonoids act as radical scavengers and are responsible for showing antioxidant activity. Due to their importance in food supplements, human health, and a considerable understanding of structure-activity relationship, they act as therapeutic agents, hence can be referred to as "nutraceuticals." *T. erecta* flower extracts possess a good amount of flavonoids and phenolics, therefore could be used as a potent source of natural antioxidant, therapeutic agent, and also food supplements. Further research work should be carried out to isolate and characterize bioactive components responsible for showing antioxidant and radical scavenging activity.

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