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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of Total Phenolic and Flavonoids Content and their Relation with Antioxidant Properties of *T. patula* flower using *In-vitro* Assay method

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ABSTRACT
The aim of this research was to isolate the total phenol and flavonoid content and evaluate the in-vitro antioxidant activity of the water flower extract of T. patula. The dry flower powder was extracted using a maceration method with distilled water and ethanol (30:70) based on green technology. The tests indicated that the extract contained a high level of total phenol and flavonoid content. The total phenolic acid equivalent was found to be 119.08±0.15, and the total flavonoid content was calculated as gallic quercetin equivalent, with a value of 71±0.22mg/g of extract. The hydroalcoholic extract was assessed for its potential antioxidant activity. The in-vitro antioxidant assay of the T. patula flowers extract demonstrated potent antioxidant activity, which was found to be superior to the standard compound ascorbic acid. The flowers extract of T. patula has the potential to be useful in the preparation of nutraceuticals and as a potent source of antioxidants for the treatment of various human diseases.
Key-Words: *in-vitro antioxidant activity methods, phenol content, flavonoid content, reducing power activity, hydrogen peroxide-scavenging activity, T. patula*

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INTRODUCTION

Now days, lots of herbaceous plants have been discovered, with active phytochemicals properties crucial for many biological activities such as antimicrobial, anti- inflammatory, antioxidant and utilized as health promoting products [1-3]. Because of many cosmetic applications found in herbs, and their different organs of plant have antioxidant and anti- inflammatory activities, preventing from aging or whitening problems of skin [4]. These activities are characterized by the presence of bioactive phenolic compounds and act as a powerful antioxidant, known to found in medicinal and ornamental plants like Tagetes, belong to Asteraceae or Compositae family [5]. Tagetes is a genus of annual and perennial herbaceous plant, normally grows as a wild and common garden plant throughout Europe and North& South America especially in Mexico, but nowadays, many species grow in tropics and subtropics region of Asian countries mainly in India and Bangladesh [6-10], even found in all around the world.

Tagetes patula L., Asteraceae, popularly known as French marigold, originated in Mexico. It is widely used as an ornamental plant and is sold freely in open markets and garden shop. In folk medicine the flowers and leaves are used for his or her antiseptic, diuretic, depurative and bug repellent activities. Chemical studies with flowers and leaves of *T. patula* identified terpenes, alkaloids, carotenoids, thiophenes, fatty acids, and flavonoids, as constituents, some of which may elicit the biological activities; these include insecticidal, nematicidal, larvicidal, antifungal, anti-inflammatory activities. The flowers of *T. patula* are a rich source of lutein and its esters. For this reason the genus is widely cultivated in Central America as food coloring, which is approved by the European Union. However, after carotenoids are extracted, the residue is discarded or only used as animal feed or fertilizer [11]. Previously reported phytochemical studies of *T. patula* suggested the presence of flavonoids and terpenes [12, 13]. Tagetes species declare as a therapeutic medicinal plant to treat various kind of diseases such as colic, diarrhoea, vomit, fever, cancer, hepatic and inflammatory disorders even in arthritis ([14, 15]. The purpose of the current study is

to investigate the antioxidant activity of *Tagetes patula* flowers, collected from Agricultural fields of SHUATS, Prayagraj Uttar Pradesh, India.

MATERIAL AND METHODS

Plant materials were collected local market from Bhopal region of Madhya Pradesh., India. Identified and authenticated was done by plants materials are verified by Pharmacognosist, Dr. Sandeep Kumar Singh, at the Central Ayurvedic Research Institute in Jhansi, Uttar Pradesh, with accession numbers CARI/H/13302021, CARI/H/13302022, CARI/H/13302023 Botanical Survey of India, Central Regional Centre, Praygraj, U.P.

Extraction of plant material

Plant material of *T. patula* was extracted separately by using cold maceration method; plant samples were collected, washed, rinsed and dried properly. Powder form of plant sample was extracted with hydroalcohalic solvent (30:70) and allows standing for 4-5 days each. The extract was filtered using filter paper to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. Extract was transferred to beaker and evaporated; excessive moisture was removed and extract was collected in air tight container. Qualitative analysis of extracts of different solvents was carried out to find out the presence of various phytoconstituents [16, 17, 18]. Extraction yield of all extracts were calculated using the following equation below:

$$\textbf{Percentage Yield} = \frac{\textbf{Actual yield}}{\textbf{Theoretical yield}} \times 100$$

Qualitative phytochemical estimation of extracts 16, 17]

Phytochemical Estimation of *T. patula* extracts were determined using standard techniques such as alkaloids, flavonoids, tannins, phenol, saponins and glycosides etc.

Total phenolic contents

Total phenolic compounds extraction was done in methanolic and water solvents and spectrophoto metrically estimated by using the Folin Ciocalteu reagent (FCR) and the results are expressed as of gallic acid equivalents per g fresh weight [19]. Experiments were performed in triplicates and results were recorded as mean ± SEM (Standard Error Mean).

Total flavonoid contents

Total flavonoids content (expressed as mg quercetin per g fresh weight) was estimated in different leaves extracts of *T. patula* flower extraction by hydroalcohalic solvents and their estimation was done by spectrophoto metrically according to Kushwaha and Verma [19]. Experiments were performed in triplicates and results were recorded as mean ± SEM (Standard Error Mean).

Evaluation of DPPH (2,2-diphenyl-1- picrylhydrazyl) Radical Scavenging Assay

Radical scavenging activity of *T. patula* was determined by following the method [20]. First of all, Prepare the 0.1mM of DPPH solution and made the extract of different concentration (25µg/ml, 50µg/ml and 100µg/ml) separately. The prepared reaction mixture contains the equal amount of DPPH solution and T. *patula* extract, and was incubate it for 30 min. at room temperature. The absorbance was recorded at 517nm against the ascorbic acid and BHT as standard. Free radical scavenging activity was calculated by using following equation:

Inhibition% = (ADPPH– A sample)/ADPPH × 100

Statistical analysis

All the experiments were conducted in triplicate manner for the determination of total phenolics, total flavonoids, and antioxidant properties using DPPH assay. All the values are expressed as mean ± standard deviation (SD).Significant differences were expressed by Correlation coefficients (r) and coefficients of determination ($r2$) by using Microsoft Excelvers. 2010. Experiments were performed in triplicates and results were recorded as mean ± SEM (Standard Error Mean).

RESULTS AND DISCUSSION

Extraction of Selected plant materials

Extraction of Selected *T. patula* was prepared by using leaves (1.90 kg), different solvent water, pet ether, chloroform and methanol. The yield of extract was found to 6.2%. The results of yield of extract indicated that highest bioactive components present in water, chloroform and methanol extract. These extract may have potential therapeutic activities.

Table 2: Extractive values obtained from *Permoterma reticulatum, Curcuma caesia* and *T. patula*

S.N.	Solvent	Color of extract	% yield
3	*T. patula*	Dark Bluish	6.2

Phytochemical screening test

According to research, PRHE has the best potential for bioactivity of any extract when it comes to glycosides, alkaloids, glycosides, phenolic compounds, tannins, saponins, flavonoids, and proteins. Hence, PRHE chose us for additional research. Results are shown in below Table 1.

Table 1: Results of phytochemical screening test of Hydroalcohalic Extract

S.N.	Constituents	*T. patula*
1	Alkaloids	+
2	Flavonoids	+
3	Phenols	+
4	Tannins	+
5	Saponins	+
6	Steroids & terpenoids	+
7	Glycosides	-
8	Carbohydrates	+
9	Anthraquinones	+

(+) indicates presence of phytochemicals; (-) indicates absence of phytochemicals

The dry flower powder was extracted using a maceration method with distilled water and ethanol (30:70) based on green technology. The tests indicated that the extract contained a high level of total phenol and flavonoid content. The total phenolic acid equivalent was found to be 119.08±0.15, and the total flavonoid content was calculated as gallic quercetin equivalent, with a value of 71±0.22mg/g of extract.

Table.1 Comparison between the Total phenolic (mgGAE/100 g) and Total Flavonoids content (mg QE/100 g) extraction from different varieties of *T. patula*

T. patula	Total phenolic compound (TPC)mg GAE/100 g Hydroalcohalic extract	Total Flavonoids compound (TFC)mg QE/100 g, Hydroalcohalic extract
Flowers	119.08±0.15	71±0.22

DPPH is a colorimetric assay, consider as efficient and best method to find out the radical scavenging activity of a concoctive antioxidant species compare to other methods. Basic principle of DPPH assay is antioxidants captures the DPPH radicals by donating the hydrogen and convert it into reduced from of DPPH-H [21, 22]. When DPPH-H is a reduced form formed, its color changes from purple to yellowish, which is recorded at 517nm by spectrophotometrically. Using DPPH assay, free radical scavenging activity of T. patula extract was determined, through which 50%inhibition concentration (IC50) of radicals was calculated. Obtained IC50 value of *T. patula* extract was in the range of 86.617µg/ml as shown in a Table 2.

Table.2 DPPH % inhibition in *Tagetus patula* varieties

SAMPLE CODE B	IC 50 (ug/ml)
25	14.4568847
50	24.67098802
75	47.84914555
100	56.0007857

IC 50 = 86.61705007

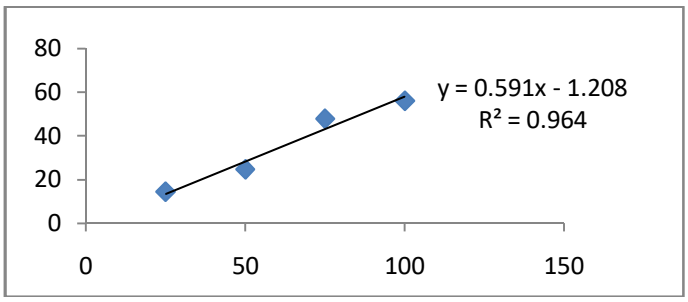

Fig.1 Correlation between antioxidant activity and water extracted total phenolic content of

This study concludes the highest phenolic and flavonoid content was observed in DY variety compare to other variety of Tagetes patula. It means DY variety had more antioxidant property. In this study, water and methanolic extract of Tagetes was compared in terms of efficacy to extract out phenolic and flavonoids compounds to a greater extent. Statistically, no significant differences were observed in the value of correlation coefficient to define the efficacy of water and methanolic extract.

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Conflict of Interest: No conflict of interest

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