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Evaluation of Total Phenolic and Flavonoids Content and their Relation with Antioxidant Properties of *Tagetes patula* Varieties, Through DPPH Assay

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A B S T R A C T

Active phytochemicals properties in *Tagetes* species. Bioactive phenolic compounds and act as a powerful antioxidant, known to found in medicinal and ornamental plants like *Tagetes*, belong to *Asteraceae* family. The observed TPC results in the range of (highest to lowest) alcoholic and water extract of Disco yellow variety (86.33mg & 78.65 mgGAE), Honey comb variety (82.00 mg &77.00mg GAE), Butter Scotch (79.65 mg & 71.65 mg GAE), Aurora orange (72.12 mg & 68.67 mg GAE).TFC was observed in the range of (highest to lowest)alcoholic and water extract of Disco yellow variety (38.0 mg & 23.53 mg QE), Honey comb variety (35.67 mg &20.66 mg QE), Aurora orange (33.23 mg & 19.67mgQE), Butter Scotch (31.46 mg &17.89 mg QE).In a more elaborate way, it could be more understandable by regression analysis demonstrate the water extracted phenolic compounds contribute maximum value i.e. about 95% (at $P< 0.05$) while methanolic extracted phenolics contribute about 92% (at$P< 0.05$) of radical scavenging properties. 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). No Significant differences were observed in the value of correlation coefficient to define the efficacy of water and methanolic extract. The purpose of the current study is to investigate the antioxidant activity of *Tagetes patula* leaves.

Introduction

Now a 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 (Achilonu *et al.*, 2018; Mehtab *et al.*, 2014; Ouelbani *et al.*, 2016). 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 (Forni *et al.*, 2019). 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 (Martha *et al.,* 2006). *Tagetes* species commonly known as marigold an herb. *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 (Kadam *et al.,* 2013), even found in all around the world.

The genera name *Tagetes* encode 33 *Tagetes* species, five of which have been introduced in an Indian garden viz. *T. erecta L.*, *T. minuta L.*, *T. patula L.*, *T. lucida* and *T. tenuifolia* (Kushwaha and Verma, 2017). Amongst these medicinal plants: (French marigold) *T. patula L.* is an aromatic annual herb belongs tothe family *Asteraceae* (Ramakrishnan *et al.*, 2015). *T. patula L.* herbaceous plant, possesses various biological properties including nematicidal (Faizi *et al.,* 2011a), antibacterial, antifungal (Faizi *et al.,* 2008), anti-inflammatory (Kasahara *et al.,* 2002), analgesic, and antioxidant (Faizi *et al.,* 2011b). Several bioactive constituents found in *T. patula L. i.e.* carotenoids (hence the orange colour), essential oils, flavonoids, sterols, tannins, saponins, triterpene alcohols, polysaccharides (Cetkovic *et al.,* 2003). Especially, the presence of the secondary metabolites was responsible for the specific kind of fragrance in *Tagetes* plants (Randon *et al.,* 2006; Hemali and Sumitra, 2014). In addition, secondary metabolites presence has an important role in biological and pharmacological activities such as anti-oxidative, anti-allergic, anti-carcinogenic, antimicrobial and hypoglycemic etc. (Stankovic, 2011). Antioxidants are free radical capturing compounds. It may protect cells from the damaging action of unstable free radicals' species (Hamid *et al.,* 2010; Stankovic, 2011). Natural antioxidant, which are obtained from plants are greater benefit in comparison to the synthetic (Rohman *et al.,* 2010)antioxidants such asbutylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), propyl gallate and tertiary butyl hydroquinone (Subhashini *et al.,* 2010). Previously reported phytochemical studies of *T. patula* suggested the presence of flavonoids and terpenes (Isman, 2006; Pavela, 2007). *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 (Vedam *et al.,* 2019; Kushwaha and Verma, 2017). The purpose of the current study is to investigate the antioxidant activity of *Tagetes patula* leaves, collected from Agricultural fields of SHUATS, Prayagraj.

Materials and Methods

Plant material

The samples of *Tagetes patula* L., were collected in October to November from the field of SHUATS, Allahabad.

Extraction of the plant material

Fresh plant samples (1g) were collected and washed properly under running water to remove the dust. Samples were grinded by using the motor and pastel and collected the extract after the centrifugation of the sample at 12,000 rpm for 15min.

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 (Kushwaha and Verma, 2017).

Total flavonoid contents

Total flavonoids content (expressed as mg quercetinperg fresh weight) were estimated in different leaves extracts of *T. patula* varieties *i.e.* (leaves extraction by methanol and water solvents) and their estimation was done by spectrophoto metrically according to Kushwaha and Verma (2017).

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

Radical scavenging activity of *Tagetes sp.*was determined by following the method (Siddhu and Saxena, 2017).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 *Tagetes* extract, then 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:

$$\text{Inhibition\%} = (A_{DPPH} - A \text{ sample})/A_{DPPH \times 100}$$

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 (r^2) by using Microsoft Excelvers. 2010.

Results and Discussion

Since the antioxidant compounds found in plants have different polarities, different solvents are used to isolate antioxidants. Water, methanol, ethanol, and acetone commonly used solvents in extraction processes (Ngo *et al.,* 2017). Phenols are very important plant constituents because of their scavenging ability for free radicals due to their hydroxyl groups presence. Hence, the phenolic compounds may be a main contributor of antioxidant action (Tosun *et al.,* 2009).

The TPC & TFC presence in the extracted samples and its yield depends on the selected solvent. The results of total phenolic and total flavonoid content were shown in Table 1. The results showed that the all plants varieties are rich sources of phenolics. TPC results were expressed as mg of gallic acid equivalent per gram fresh weight (mgGAE/100g). The observed TPC results in the range of (highest to lowest) alcoholic and water extract of Disco yellow variety (86.33mg & 78.65 mgGAE), Honey comb variety (82.00 mg &77.00mg GAE), Butter Scotch (79.65 mg & 71.65 mg GAE), Aurora orange (72.12 mg & 68.67 mg GAE). Similar finding was observed by Kushwaha and Verma (2017), reported 30 mg GAE/g and 80mg GAE / gcontent was found in aerial part (flower and leaves respectively) of *T. patula* while highest phenolic content was reported in methanolic extract (49.764 mg GAE/g) of *T. erecta* flower and lowest content was found in chloroform extract (15.450) by Siddhu and Sexena (2017).

TFC was observed in the range of (highest to lowest) alcoholic and water extract of Disco yellow variety (38.0 mg & 23.53 mg QE), Honey comb variety (35.67 mg & 20.66 mg QE), Aurora orange (33.23 mg & 19.67mgQE), Butter Scotch (31.46 mg &17.89 mg QE). Previously reported studies confirm that the phenolics compounds had antioxidant and free radical species scavengers' properties supported by (Huyut *et al.,* 2017; Leopoldini *et al.,* 2017). Phenolics

antioxidants act as reducing agents, hydrogen donors and reactive oxygen capturer (Kasote *et al.,* 2015).Instead of antioxidant property, phenolics compounds gaining more interest among researchers because of nutritious values, actually, derived from naturally agricultural crops and vegetables (Krishnaiah *et al.,* 2011).

In addition to this, these compounds impart stabilizing nature to biological membrane lipids against peroxidation and stops the reactive oxidizing enzymes (Kurutas,2016; Parthasarathy *et al.,* 2009). An effective reason behind this antioxidant activity, is to provide a stability to a cellular membrane by phenolics compounds such as flavonoids, its structure altered and their functional groups substitution mainly-OH groups and other groups contribute also, thereby, affecting flavonoids antioxidant properties (Forni *et al.,* 2019).

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 (Aksoy *et al.,* 2013; Sahu *et al.,* 2013). When DPPH-H 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 39.732µg/ml to 16.665µg/ml, as shown in a Table 2.

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*

***Tagetuspatula* Varieties**	**Total phenolic compound (TPC)mg GAE/100 g**	**Total phenolic compound (TPC)mg GAE/100 g**	**Total Flavonoids compound (TFC)mg QE/100 g**	**Total Flavonoids compound (TFC)mg QE/100 g**
	Methanolic extract Mean±S.D	Water extract Mean±S.D	Methanolic extract Mean±S.D	Water extract Mean±S.D
HC	82±0.45	77.0±0.29	35.67±0.29	20.66±0.86
DY	86.33±0.21	78.65±.0.26	38.0±0.24	23.53±0.76
BS	79.65±0.22	71.11±0.39	31.46±0.19	17.89±0.77
AO	72.12±0.16	68.67±0.25	33.23±0.21	19.67±0.72

Butter Scotch: BS, Honey comb HC, Disco yellow DY, Aurora orange: AO

Table.2 DPPH % inhibition in *Tagetuspatula*varieties

***Tagetuspatula*varieties**	**DPPH(%) of inhibition**
HC	35.975
DY	39.732
BS	16.665
AO	36.332

Butter Scotch: BS, Honey comb HC, Disco yellow DY, Aurora orange: AO

Fig.1 Correlation between antioxidant activity and water extracted total phenolic content of *T. patula* varieties

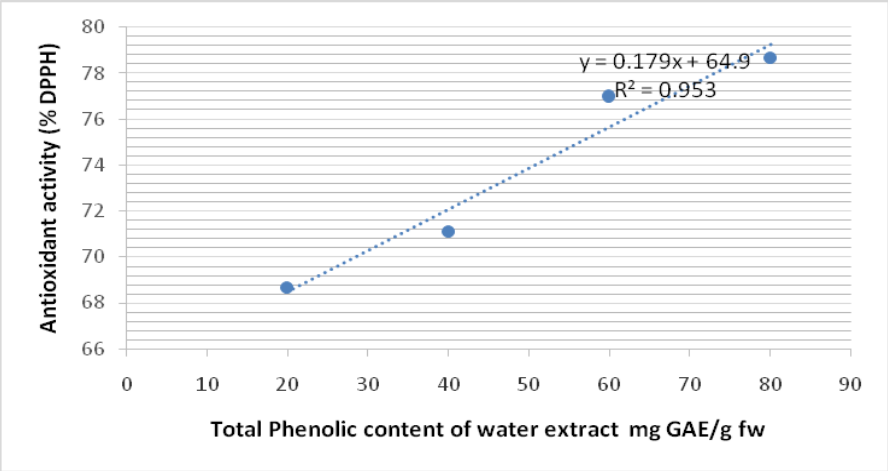
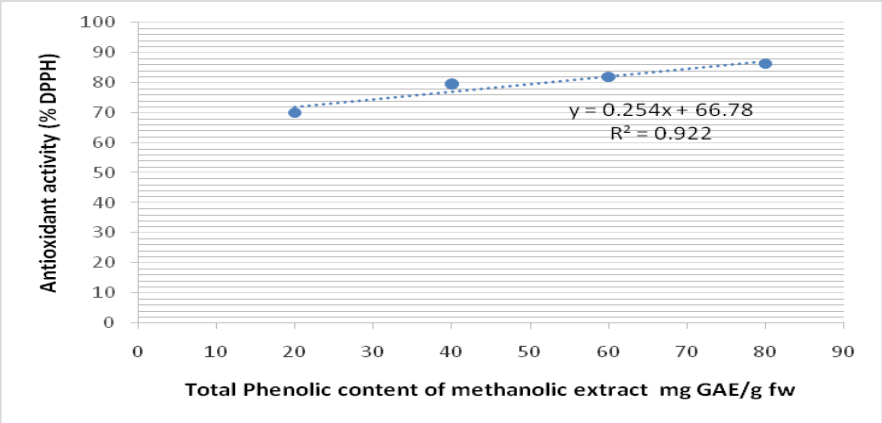


Fig.2 Correlation between antioxidant activity and water extracted total phenolic content of *T. patula* varieties



The obtained results showed the relation between the antioxidant activity and phenolic content determined by using the DPPH assay applied on *T. patula* varieties extract as shown in Figures 1 and 2, respectively. In a more elaborate way, it could be understandable by regression analysis demonstrate the water extracted phenolic compounds contribute maximum value i.e. about 95% (r^2= 0.9537 at $P < 0.05$) while methanolic extracted phenolics contribute about 92% (r^2=0.9222 at $P < 0.05$) of radical scavenging properties respectively as shown in (Fig. 1, 2). Remaining antioxidant property is contributed by other non-phenolics compounds such as vitamins and carotenoids (Pourmorad *et al.*, 2006). The major antioxidants compounds like phenolics and flavonoids plays as a primary antioxidant (Chang *et al.*, 2002; Kulma *et al.*, 2014)because it is known to react with -OH, $O_2^{-2}$and lipid peroxide reactive species (Munhoz *et al.*, 2014; Kushwah and Verma 2017). These antioxidant species are known to protect the DNA from oxidative stress, inhibit the tumoric cell growth, and had anti-inflammatory and antimicrobial activities as well. Present study was supported by Yao *et*

al., (2010), there study showed the significant difference between the antioxidant activity, total phenolics and total flavonoids content found in celery and set correlation r value was positive, it means there was a direct relation between them.

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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