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SCREENING OF *Tagetes minuta* ETHANOLIC EXTRACT FOR ITS PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT PROPERTY, TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENTS

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ABSTRACT
Medicinal plants are widely distributed in Jammu and Kashmir and their biological role and phytochemical composition are not still fully reconnoitered. The present work was undertaken to explore the preliminary phytochemical screening and antioxidant value of ethanolic extract of *Tagetes minuta* (EETM). The study was done by using various *in vitro* methods such as, Preliminary qualitative phytochemical assay, DPPH radical scavenging activity, reducing power assay and, Total phenolic and Total flavonoid content estimations. The whole EETM showed a fair number of phytochemicals with high free radical scavenging activity as evidenced by low IC_{50} in DPPH (40.77 µg/ml) and increase in absorbance in case of reducing power assay. The TPC and TFC of the extract were found to be was 130.8±0.6 mg/g Gallic acid equivalent and 57.33±2.51 mg/g Rutin equivalent of the extract respectively. The findings from the present investigation clearly showed that EETM possessed high phenolic and flavonoid contents and potential antioxidant activity and could be used as a valuable source of natural antioxidants and hence can prevent the body from diseases associated with oxidative stress.

KEYWORDS: *Tagetes minuta*,Gallic acid, Rutin and Antioxidants.

INTRODUCTION
Medicinal plants as sources of bioactive constituents have been used traditionally to cure various ailments since time immoral in Ayurveda, Unani and Siddhi system of medicines. However, during past few years, synthetic therapeutic agents occupy the position for curing various diseases, but, due to their side effects, researchers are now converging to explore the potentiality of traditional medicines due to their natural origin, cost-effectiveness and lesser side effects.[1] Interest in medicinal plants as a re-emerging health aid in the maintenance of personal health and well-being has been fueled by rising costs of prescription drugs and the bioprospecting of new plant-derived drugs.[2] At present near about 25% of active compounds derived from plants are used as prescribed medicines.[3] The most relevant and practical way to fight against degenerative diseases is to strengthen antioxidant defense system of our body and that could be achieved by consumption of vegetables, fruits, and edible plants.[4] The importance of natural antioxidants has been clarified by numerous studies which have demonstrated that the consumption of foods rich in such phytochemicals can exert beneficial effects upon human health, possibly by interfering with the processes involved in reactive oxygen and nitrogen species-mediated pathologies. This has resulted in a resurgence in phyto-pharmacognosy with extensive attention upon the role that plant secondary metabolites may have in preventative medicine.[5,6] *Tagetes minuta* also known as southern cone marigold, stinking roger or black mint, is a tall upright marigold plant from the genus *Tagetes*. It is an erect, woody annual herb, usually 0.5-2 m tall with strongly odorous foliage. The strong smelling essential oils of *T. minuta* have enabled it to be used for many purposes, including as a relish, laxative, diuretic, flavouring, insect repellent, stimulant and snuff.[7]

As there is no comprehensive report on *in vitro* antioxidant activity of *Tagetes minuta*, an attempt has

been made to explore *in vitro* antioxidant data of *Tagetes minuta* which is essential for its pharmacological studies.

MATERIALS AND METHODS

Collection of plant material

The whole plant of *Tagetes minuta* was purchased from Indian Institute of integrative medicines Srinagar J&K, India. The plant material was washed with double distilled water and thereafter shade dried for the period of 2 weeks at room temperature.

Chemicals

All chemicals, solvents used were of analytical grade purchased from Merck, Mumbai and HiMedia, Mumbai.

Preparation of extract

The fully dried plant material was powered with the help of mechanical grinder. The powder was extracted in 90% ethanol by using the Soxhlet extractor. The percentage yield of the extract was determined by using the formula given below. The ethanol extract was then dried under vacuum and the semi-solid material thus obtained was stored in storage vials which were kept at -4°C for further use.

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material used}} \times 100$$

Prepared extracts were observed for colour, odour, and texture, and were packed in airtight bottles and labeled for further proceedings.

Solubility test of Extract

The solubility of whole plant ethanolic extract of *Tagetes minuta* was observed in different solvents.

Phytochemical investigation of crude extract of *Tagetes minuta*

Phytochemical screening of the extract was carried out according to the standard procedures [8, 9]. EETM was subjected to preliminary phytochemical screening to identify the various phytoconstituents present i.e. alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, saponins, and tannins.

In vitro Antioxidant Assay

DPPH radical scavenging activity[10]

0.1 mM DPPH solution (4 mg/100 ml) was prepared in methanol. Different concentrations of the test sample with methanol were prepared. To the 2 ml of the test sample was added 1ml of DPPH solution. The mixture was incubated at room temperature for 10 minutes. The absorbance was taken at 515 nm against a blank (methanol). Percentage Inhibition was calculated by using following formula: -

% Inhibition = [(AC 515 nm- AS 515 nm/ AC 515 nm) x 100].

A curve for % Inhibition and concentration was plotted and IC_{50} was estimated by using a line of regression.

Reducing Power Assay[11]

Compounds with reducing power indicate that they are electron donors and associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity.[12] The higher the absorbance of the reaction mixture, the higher would be the reducing power.

Different concentrations of test sample were prepared. Added 0.5 ml of different concentrations of sample with 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 0.5ml of potassium ferricyanide (0.5ml, 1%W/V). The reaction mixture was incubated at 50° C for 20min. After cooling, 1.5 ml of trichloroacetic acid solution (10% W/V) was added to terminate the reaction. 0.5 ml ferric chloride (0.1% W/V) was added and absorbance was measured at 700nm. The curve between absorbance and concentration was plotted. Increased absorbance of the reaction mixture indicated an increase in reducing power.

Total Phenolic Content Estimation[13]

Different concentrations of Gallic acid (10 to 100µg/ml) in methanol were prepared. The test sample was prepared in methanol or, the solvent of near about same polarity (100 µg/ml).0.5 ml of different concentrations of Gallic acid/ test sample with 2 ml Folin-Ciocalteu reagent (1:10 in deionized water) was added. Also, 4 ml of sodium carbonate solution was added to the resulting solution. The testing mixture was incubated at room temperature for 30 minutes with intermittent shaking. The absorbance was taken at 765 nm (due to developed blue colour) using methanol as blank. A standard curve of different concentrations of Gallic acid was prepared and line of regression was found. The absorbance of the test sample was put in the line of regression of standard curve of Gallic acid. Total phenolic content was calculated and expressed as mg/gm or µg/mg Gallic acid equivalent.

Total Flavonoid Content Estimation[14]

Different concentrations of Rutin (10 to 100µg/ml) in methanol were prepared. The test sample was prepared in methanol or solvent of near about same polarity (100µg/ml). 0.5 ml aliquots of the appropriately diluted sample solution with 2 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution were mixed. After 6 minutes, 0.15 mL of a 10% $AlCl_3$ solution was added and allowed to stand for 6 minutes, then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and then mixed the mixture thoroughly and allowed to stand for another 15 minutes. The absorbance of the mixture at 510 nm was taken using water as the blank. The standard curve for different concentration of Rutin was prepared and line of regression was drawn. Total flavonoid content was calculated and expressed as mg/g or, µg/mg Rutin equivalent.

RESULTS AND DISCUSSION

The plant material was washed with double distilled water and shade dried at room temperature for the time period of 15 days. The dried plant material was grinded with the help of mechanical grinder. The loss of weight on drying of the whole plant of *Tagetes minuta* was 67.25% and its yield was 6.6%. *Tagetes minuta* extract was soluble in methanol, pet ether, chloroform, ethanol, ethyl acetate and DMSO but was found partially soluble in water and acetone.

Observations of phytochemical investigation

The phytochemical analysis of an ethanolic extract of *Tagetes minuta* showed the presence of alkaloids, terpenoids, flavonoids, carbohydrates, glycosides, tannins, phenolic compounds, saponins and amino acids.

Antioxidant activity of EETM

DPPH assay is one of the most reliable method for determination of antioxidant activity of plant extracts. Our results of DPPH assay showed that EETM possesses strong antioxidant property.

The EETM confirmed H-donor ability and can serve as a free radical inhibitor or exhibit significant DPPH radical inhibition. The IC_{50} value obtained for EETM was 40.77 µg/ml (Table: 1 and Fig: 1) and its scavenging effect was compared with standard ascorbic acid with IC_{50} 37.03 µg/ml (Table: 2 and Fig: 2). While donating hydrogen to DPPH free radical which is deep violet in colour gets converted into α, α-diphenyl-β- picrylhydrazine colorless which indicates the antioxidant potential of plant extract [15]. The scavenging activity of free radicals is directly linked to the amount of phenolics.[16,17] The results of our study were in a compliance with the antioxidant activity of *Taraxcum officinale*[18], *Iris kashmiriana* which was concentration dependent on plant sample.[19]

Another method used for determination of antioxidant activity of EETM was reducing power assay. For reducing power assay, higher the absorbance of the reaction mixture, higher would be its reducing power (Table:3). The results were in acquiescence with the *Rosmarinus officinalis*[20], *Coleus ambonicus*[21] whom also worked on reducing power assay of said plant extracts.

Total Phenol and Total Flavonoid Content

Different concentrations of Gallic acid were made (Table: 4). Absorbance was taken at 765 nm using methanol as blank so to draw the standard curve of Gallic acid (Fig. 3). The total phenolic content in EETM was 130.8±0.6 mg/g Gallic acid equivalent (Table: 5).

Different concentrations of Rutin were made and absorbance was taken at 510 nm with water as blank (Table: 6) and a standard curve was plotted (Fig. 4). The total flavonoid content of EETM was 57.33±2.51 mg/g Rutin respectively (Table: 7).

Phenolic compounds showed a wide range of distribution. Their diverse biological and chemical activities like antioxidant activity, scavenging active oxygen species as well as electrophiles and hamper the nitrosation[22] and metal chelating activity, autoxidation and hasten the cellular enzymatic activities[23] made them one of the important naturally occurring antioxidants. Furthermore, the reduction potential of flavonoid phenoxy radicals ranges between 540±700 mV. The analogous parent flavonoids are anticipated to competently inactivate diverse reactive oxygen species with higher potentials of 2000±950 mV.[24,25] The main cause of development of the various disease is the damaging activity of reactive oxygen species such as superoxide free radical, hydroxyl radical, singlet oxygen and hydrogen peroxide.[26] Therefore, flavonoids too act as splendid antioxidants and natural drugs for the treatment of these diseases.

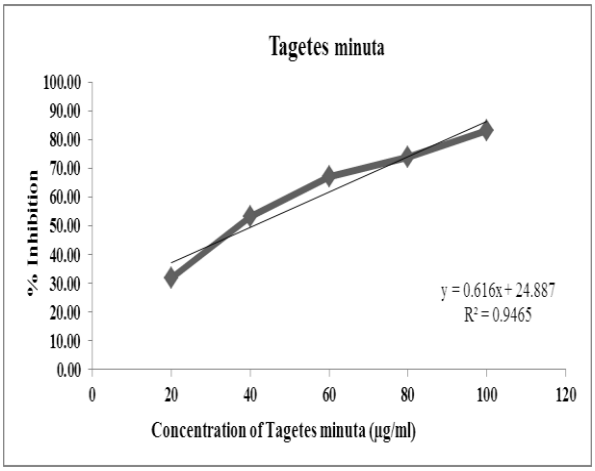

Fig. 1 - Represents the regression curve of ethanolic whole plant extract of *Tagetes minuta* by DPPH assay method.

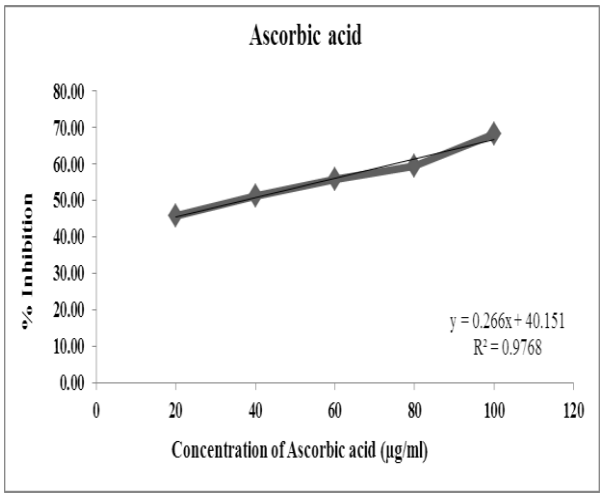

Fig. 2 – Represents the regression curve of Ascorbic acid by DPPH assay method.

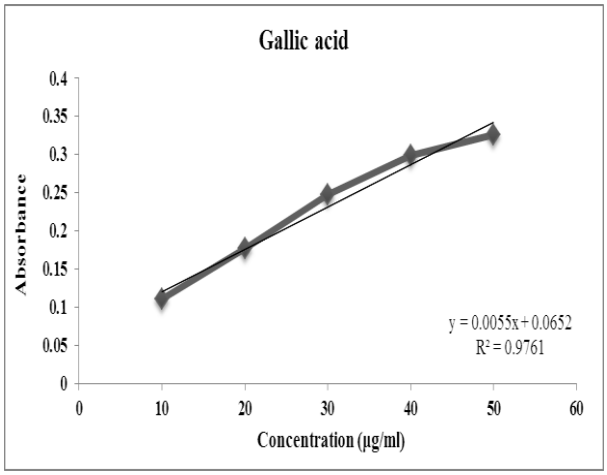

Fig. 3 – Represents the regression curve Gallic acid for total phenolic content.

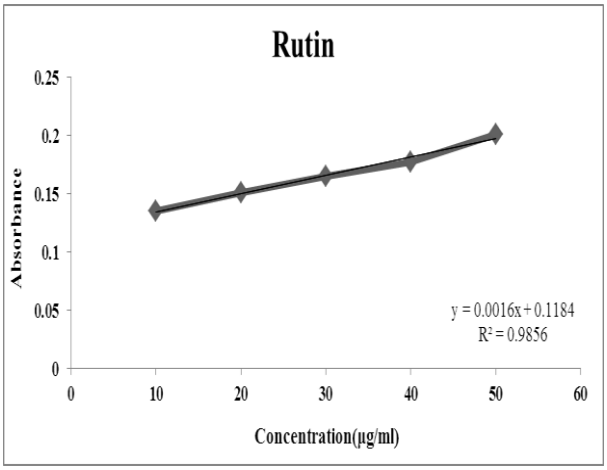

Fig. 4 – Represents the regression curve of Rutin for Total flavonoid content.

Table: 1 - Showing the % Inhibition of DPPH by ethanolic extract of *Tagetes minuta.*

S. No.	Conc. (mg/ml)	Absorbance (Control), A_c	Absorbance (Test), A_t	% Inhibition	IC_{50} (µg/ml)
1	20	0.530	0.362	0.31698	40.77 µg/ml
2	40		0.247	0.53396	
3	60		0.174	0.6717	
4	80		0.138	0.73962	
5	100		0.090	0.83019	

Table: 2 - Showing the % Inhibition of DPPH by Ascorbic acid.

S. No.	Conc. (mg/ml)	Absorbance (Control), A_c	Absorbance (Test), A_t	% Inhibition	IC_{50} (µg/ml)
1	20	0.530	0.287	0.45849	37.03 µg/ml
2	40		0.259	0.51132	
3	60		0.234	0.55849	
4	80		0.215	0.59434	
5	100		0.168	0.68302	

Table: 13– Showing the Reducing Power of ethanolic extract of *Tagetes minuta.*

S. No.	Concentration µg/ml	Absorbance
1	10	0.088
2	20	0.128
3	40	0.181
4	60	0.248
5	80	0.318
6	100	0.374

Table: 4 - Showing different absorbance of Gallic Acid at different concentrations.

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.1098
2	20	0.1763
3	30	0.2468
4	40	0.2981
5	50	0.3258

Table: 5 - Showing Total Phenolic Content in whole plant ethanolic extract of *Tagetes minuta*.

S. No.	Concentration	Absorbance	Total Phenolic content in mg/g Gallic acid equivalent
1	1mg/ml	0.719	130.8
2	1mg/ml	0.722	131.4
3	1mg/ml	0.716	130.2
Mean±SD		**0.719±0.003**	**130.8±0.6**

Table: 6 - Showing different absorbance of Rutin at different concentrationsTotal flavonoid content.

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.136
2	20	0.152
3	30	0.163
4	40	0.177
5	50	0.198

Table: 7 - Showing total flavonoid content in ethanolic extract of *Tagetes minuta* whole plant.

S. No.	Concentration	Absorbance	Total Flavonoid content in mg/g Rutin equivalent
1	1mg/ml	0.178	60
2	1mg/ml	0.173	55
3	1mg/ml	0.175	57
MEAN±SD			**57.33 ± 2.51**

CONCLUSION

The present study evidence indicated that the plant *Tagetes minuta* is an opulent source of active phytoconstituents responsible for pharmacological activities. The results of present study suggest that tested plan extract has effective antioxidant activity and free radical scavenging property. Nevertheless, the great antioxidant potential will be of immense benefit from the consumption of this medicinal plant.

Conflict of interest statement

The authors declare no conflict of interest.

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