



Evaluation of phytochemicals, antioxidants and anti-inflammatory activity of fresh leaf and flower extracts of *Tagetes erecta* L. grown in Kalaburagi

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Manuscript details:

Received : 16.01.2018
Revised : 09.03.2018
Accepted : 29.03.2018
Published : 24.04.2018

Editor: Dr. Arvind Chavhan

Cite this article as:

Rathod Manjula A and Mathad Pratima (2018) Evaluation of phytochemicals, antioxidants and anti-inflammatory activity of fresh leaf and flower extracts of *Tagetes erecta* L. grown in Kalaburagi., *Int. J. of Life Sciences*, Volume 6(2): 427-433

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Available online on
<http://www.ijlsci.in>
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

Tagetes erecta L. (Asteraceae) is used to treat epileptic fits (Ayurveda), fever, wounds and microbial infections of livestock's in folklore medicine of Kalaburagi district. As little work has been carried out on fresh material so present study aims to evaluate aqueous extracts of fresh leaf and flower for phytochemicals, antioxidants and anti-inflammatory activities. The results revealed that the leaf extract was rich in primary as well as secondary metabolites as compared to flower extract. The physicochemical analysis such as moisture, crude fat, crude fibre content and total ash values found higher in leaf extract than flower extract. The DPPH and Nitric oxide radical scavenging activity found higher in flower extract than the leaf extract when compared to standards. Whereas the Hydrogen peroxide and Reducing power assay of leaf extract showed higher activity than flower extract at different concentrations. Anti-inflammatory activity showed that the HRBC membrane stabilization activity of leaf extract was found higher than flower extract and albumin denaturation activity of flower extract was found higher than leaf extract. From the study it is evident that the fresh leaf and flower extracts of *Tagetes erecta* are effective source of naturally and pharmacologically active compounds which can be used in herbal remedies.

Key words: Phytochemicals, Free radicals, proximate analysis, anti-inflammatory *Tagetes erecta*, leaf, flowers, Kalaburagi.

INTRODUCTION

India is a land where various traditional medicinal systems together exists, all the medicinal systems are plants based. The medicinal value of plants lies in some chemical substances that have a definite physiological functions in the human body (Mathad *et al.* 2016).

According to WHO herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries (Hussain and Kumarsen 2014). The beneficial effect of herbal medicine typically result from the combination of secondary metabolites produced in the herbs such as alkaloids, flavonoids, phenols, tannins, glycosides and gums etc (Gayathri *et al.* 2015). An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells (Shetty *et al.* 2015). Many plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxynitrite which results in oxidative stress leading to cellular damage (Bjelakovic *et al.* 2013). Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as the increase of vascular permeability, increase of protein denaturation and membrane alteration. Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation (Summner *et al.* 2000). *Tagetes erecta* L. Marigold (Asteraceae) is commonly known as 'Genda phul' in India. It is stout, branching herb, native to Mexico and other warmer parts of America and naturalized elsewhere in the tropics and subtropics including Bangladesh and India. These are erect, rapid growing annual herbs with yellow and orange flowers. Numerous traditional uses of this plant have been reported in

medicine as well as cosmetics. The whole plant has been used to treat bronchitis, rheumatic pain, fever, cold, respiratory diseases, muscle relaxer and as a stimulant. The leaves are used to treat microbial infection, wound healing, fever and epileptic fits (Ayurveda). The flowers have been used to treat fevers, scabies, liver complaints, eye diseases, astringent, carminative and stomachic effects (Arefin *et al.* 2015). Gulbarga (Kalaburagi) district one of the 30 districts of Karnataka state in southern India. The city is drought prone area, climate is very hot in summer with temperature ranging up to 45°C to 46°C and this temperature adversely affects the metabolism of plants (The Hindu 2011). *Tagetes erecta* which is a natural wound healer used to treat microbial infections of livestock's in folklore medicine (fresh material) of Kalaburagi district. So the present study aims to evaluate fresh leaf and flower extract to standardize the plant on the bases of phytochemicals, antioxidants and anti-inflammatory activities.

MATERIAL AND METHODS

Collection and identification of the plant

The *Tagetes erecta* L. is collected from the various places of Kalaburagi district. The plant was identified using various floras available in the Department of P.G. Studies and Research in Botany, Gulbarga University, Kalaburagi.

Extraction of plant material

Fresh material was extracted using dist. water (aqueous) with the pestle and mortar. The extracts were taken in a conical flask and kept on shaker for 24 hrs. Then the extracts were filtered using Whatman No.1 paper, which is used for the different analysis.



Fig (a) *Tagetes erecta* Leaf



***Tagetes erecta* Fig (b) Flower**

Physicochemical (Proximate) analysis

Proximate analysis of a substance constitutes different classes of nutrients present in samples such as moisture content, dry matter content, crude fat content, crude fibre content, total ash value, acid insoluble ash value and water soluble ash values are determined by using standard methods.

Quantitative estimations of primary metabolites

1. Estimation of total carbohydrates (Yemm and Willis, 1954)
2. Estimation of proteins (Lowry, 1951)

Quantitative estimation of secondary metabolites

1. Estimation of Flavonoids (Swain And Hill, 1959)
2. Estimation of Alkaloids (Horn Borne, 1973)
3. Estimation of total phenols (Bray and Thorpe, (1964)
4. Estimation of Tannins (Shreelalitha, 2016)
5. Estimation of Terpenoids (Theng and Korpenwar 2013)

Determination of in-vitro antioxidant and anti-inflammatory activity

DPPH radical scavenging activity

The DPPH (2, 2 diphenyl-1-picrylhydrazyl) radical scavenging activity was carried out following the procedure of Vishnuvathan *et al.* (2017). 2ml of various concentrations such as 0.2, 0.4, 0.6, 0.8, 1 and 2mg of (test sample) in aqueous extract were prepared to which 1ml of 0.1 mM of DPPH in methanol solution is added. After 30 minutes of incubation period, the absorbance was measured at 517nm. The free radical scavenging activity of each sample was determined by comparing its absorbance with that of a blank solution (Control). The Ascorbic acid and Butylated hydroxyl anisole were used as standards. The DPPH radical scavenging activity was calculated using the following equation. % of inhibition = $(Ac - As)/Ac \times 100$. Where Ac is the absorbance of the control and As is the absorbance of test the sample.

Nitric oxide radical scavenging activity

Nitric oxide scavenging activity was carried out following the procedure of Vishnuvathan *et al.* (2017). 0.5ml of 0.1M PBS (pH7.4) is added to the 2ml of 10 mM Sodium nitroprusside and mixed well. To this mixture various concentrations of plant extract such as 0.2, 0.4, 0.6, 0.8, 1 and 2mg were added and incubated for 160 min at 30°C. After incubation period, its absorbance was measured at 546 nm. The nitric oxide radical scavenging activity of each sample was determined by comparing its

absorbance with that of a blank solution. Ascorbic acid and Phloroglucinol taken as standards. The percentage of inhibition of nitric oxide radical by extracts was calculated by using following formula. % of inhibition = $(Ac - As)/Ac \times 100$. Where Ac is the absorbance of the control and As is the absorbance of the test sample.

Hydrogen peroxide assay

Hydrogen peroxide radical scavenging activity was carried out following the procedure of Vishnuvathan *et al.* (2017) with little modification. A solution of H₂O₂ (30 mM) is prepared in distilled water. The plant extract of different concentrations such as 0.2, 0.4, 0.6, 0.8, 1 and 2mg in 2ml phosphate buffer (0.1 M, pH 7.4) mixed well and 0.5 µl of H₂O₂ (30 mM) solution is added. After 10 minutes the absorbance of the reaction mixture was recorded at 230 nm. The hydrogen peroxide scavenging activity of each sample was determined by comparing its absorbance with that of a blank solution. The % of inhibition of hydrogen peroxide radical by extracts was calculated by using following formula, H₂O₂ scavenging activity (%) = $(Ac - As) / Ac \times 100$. Where Ac is the absorbance of the control and As is the absorbance of the sample.

Reducing power assay

Reducing power assay of the extract was evaluated according to the protocol of (Jayanthi P and Lalitha P. (2011). The 1ml of different concentrations of aqueous extract such as 0.2, 0.4, 0.6, 0.8, 1 and 2 mg were mixed with 0.1M phosphate buffer (pH6.6) and potassium ferricyanide (1ml, 1%), and the mixture was incubated at 50°C for 20 min. Next 2ml of Trichloroacetic acid (10%) is added to the reaction mixture, and then centrifuged at 10000 RPM for 10 min. The upper layer of the solution (1ml) is mixed with distilled water (1ml) and ferric chloride (150 µl, 0.1%), and the absorbance was measured at 700nm against the blank sample (Control). The test was performed in triplicates and results are recorded.

In-vitro anti-inflammatory activity of plant extracts were evaluated by following methods

The Human red blood cell (HRBC) membrane stabilization method.

Blood sample (2ml) was collected from a volunteer in a heparinized tube and washed with phosphate buffered saline twice and centrifuged at 3000 rpm for 10 min. Then, RBC was suspended in normal saline and taken in a tube (0.5 ml) with 0.5 ml of extract and 0.5 ml

hypotonic solution and incubated for 30 min at room temperature. Then, the contents were centrifuged at 1500 rpm for 10 min and the supernatant was collected and the absorbance read at 560nm. Based on the absorbance of extract and control, the membrane stabilization effect was calculated.

Inhibition of albumin denaturation method.

The reaction mixture was consisting of plant extracts and 1% aqueous solution of bovine albumin fraction pH, of the reaction mixture was adjusted with 1N HCL. The extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min, after cooling the samples the turbidity was measured by spectrophotometrically at 660nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows,

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control} \times 100}$$

Statistical analysis

The results were expressed as mean \pm Standard error mean. (Significant value $P < 0.0001$) using one way ANOVA (Graph Pad InStat3) and Microsoft excel.

RESULTS AND DISCUSSION

In the present study aqueous extract of fresh leaf and flower of *Tagetes erecta* are subjected to phytochemicals, antioxidants and anti-inflammatory activities. The results revealed that the leaf extract was found rich in primary as well as secondary metabolites as compared to flower extract and also proximate analysis such as moisture content, dry matter, crude fat content, crude fibre, total ash value, acid insoluble ash value, water soluble ash value in leaf extract found higher than flower extract. The results of antioxidants study showed that the DPPH and Nitric oxide radical scavenging activity were higher in flower extract than the leaf extract when compared to standards. The Butyl hydrated anisole ascorbic acid and Phloroglucinol taken as standards in Fig 2 and 3. It is interesting to note that the Hydrogen peroxide assay and Reducing power assay of leaf extract showed higher activity than flower extract at different concentrations and these values are very near to standard ascorbic acid Fig No.4 and 5. Anti-inflammatory activity showed that the HRBC membrane stabilization activity of leaf extract was found higher than flower extract and standard dichlorofenac in Fig No. 6 whereas albumin denaturation activity of flower extract was found higher than leaf extract as well as standard in Fig No 7.

Table 1. Preliminary screening of primary and secondary metabolites

Sr. No.	Phytoconstituents	<i>T. erecta</i> Leaf extract					<i>T. erecta</i> flower extract				
		E ace.	Pet-eth.	Ace.	Eth.	Aq.	E ace.	Pet-eth.	Ace.	Eth.	Aq.
1	Carbohydrates	+	+	+	+	+	+	+	+	+	+
2	Lipids	+	+	+	+	+	+	+	+	+	+
3	Proteins	+	+	+	+	+	+	+	+	+	+
4	Alkaloids	+	+	+	+	+	+	+	+	+	+
5	Flavonoids	+	+	+	+	+	+	+	+	+	+
6	Phenols	+	+	+	+	+	+	+	+	+	+
7	Tannins	+	+	+	+	+	+	+	+	+	+
8	Glycosides	-	-	-	-	-	+	+	+	+	+
9	Lignin's	-	-	-	-	-	-	-	-	-	-
10	Saponins	+	+	-	+	+	+	-	-	+	+
11	Terpenoids	+	+	+	+	+	+	+	+	+	+
12	Steroids	-	-	-	-	-	-	-	-	-	-

*Ethyl acetate, Petroleum ether, Acetone, Ethanol and Aqueous extracts. , + Present and - Absent.

Table 2. Quantitative estimation of primary metabolites

Sr. No.	Primary metabolites	<i>T. erecta</i> leaf ext.	<i>T. erecta</i> flower ext.
1	Carbohydrates	752.6 \pm 0.01	532.4 \pm 0.00
2	Proteins	313.3 \pm 0.02	234.5 \pm 0.00

Mean \pm Standard error mean, Significant value $P < 0.0001$.

Table 3. Quantitative estimation of secondary metabolites (mg/100g)

Sl No.	Secondary metabolites	<i>T erecta</i> leaf ext.	<i>T erecta</i> flower ext.
1	Alkaloids	405.5±0.00	292.0±0.01
2	Flavonoids	447.5±0.02	244.0±0.00
3	Phenols	930.0±0.01	480.0±0.21
4	Tannins	425.0±0.00	380.0±0.01
5	Terpenoids	365.3±0.00	263.7±0.00

Mean ± Standard error mean, Significant value P < 0.0001.

Table No. 4. Proximate analysis of *Tagetes erecta* leaf and flower extract (in %).

Parameters	Moisture Content	Dry Mater	Total ash value	Water Soluble Ash Value	Acid Insoluble Ash value	Crude Fibre Content	Crude Fat Content
<i>T erecta</i> leaf ext.	83.88%	16.12%	1.42%	79.1%	3.16%	21.44%	1.22%
<i>T erecta</i> flower ext.	85.50%	10.3%	0.87%	56.7%	2.56%	18.34%	0.53%

Mean ± Standard error mean, Significant value P < 0.0001.

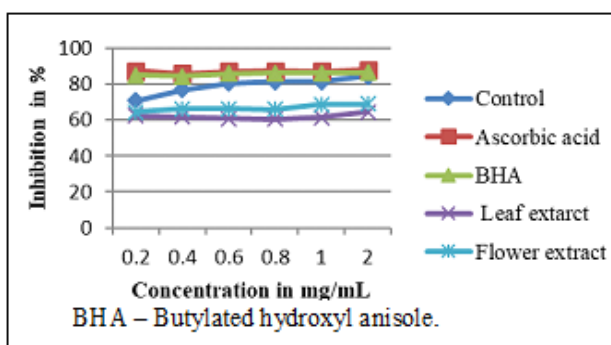


Fig. 1. DPPH radical scavenging activity

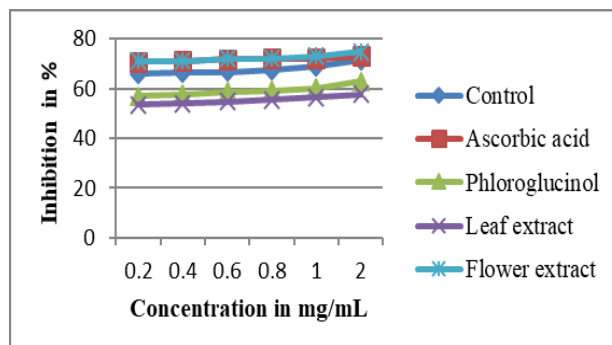


Fig. 2. Nitric oxide scavenging activity

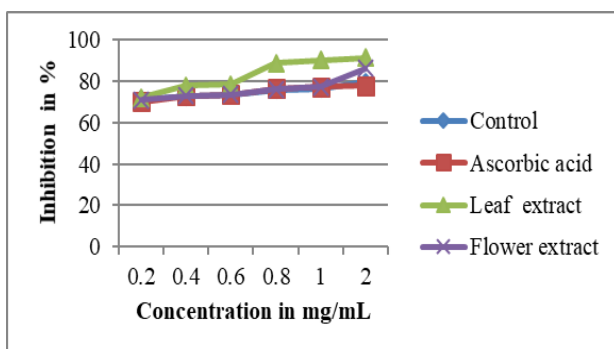


Fig. 3. Hydrogen peroxide assay

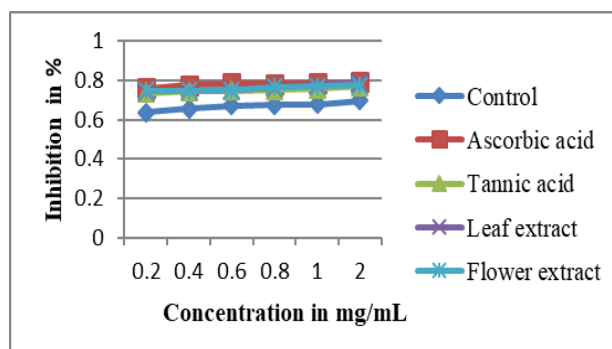


Fig. 4. Reducing power assay

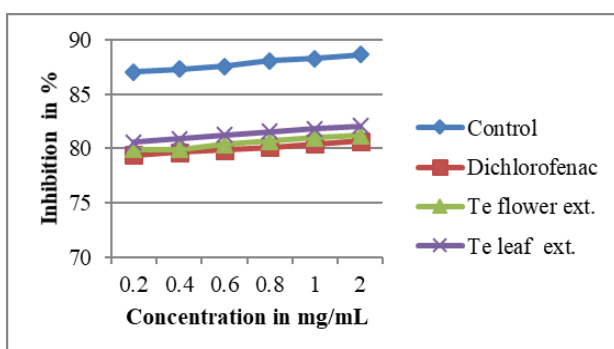


Fig. 5 HRBC membrane stabilization method

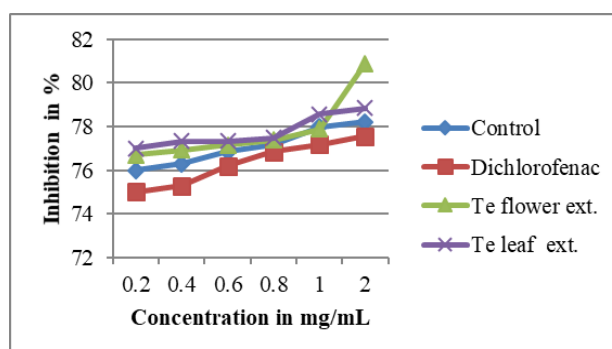


Fig. 6 Inhibition of albumin denaturation method

DISCUSSIONS

Present study showed that the aqueous extracts of fresh leaf and flower of *Tagetes erecta* are rich in phytochemicals, antioxidants as well as anti-inflammatory activities. Different phytochemicals have been found to possess a wide range of medicinal properties, which may help in protection against various diseases. For example, according to the literatures and several studies alkaloids protect against chronic diseases. Steroids and terpenoids show the analgesic properties (Mathad *et al.* 2016). Medicinal plants derivatives characterized by secondary metabolites are widely used for medicinal purposes are becoming popular all over the world as a natural alternative to synthetically produced chemicals both in traditional and allopathic system of medicine (Shetty *et al.* 2015). Nitric oxide is a very unstable species, so under aerobic condition it can react with O₂ to produce its stable products such as nitrate and nitrite through intermediates Hydroxyl radical is an extremely reactive species formed in biological systems. It is capable of damaging almost every molecule found in living cells (Prabha and Gopalakrishnan. *et al.* 2015). HRBC membrane is similar to the lysosomal membrane. During inflammation, histamine from damaged tissues makes capillaries more permeable and lysosomes of damaged cells release their enzymes which help breakdown damaged tissue but may also cause destruction of nearby healthy tissue (Dharsana and Molly Mathew SR (2015). The extensive literature survey revealed that *Tagetes erecta* is important medicinal plant with diverse pharmacological spectrum. The plant shows the presence of many chemical constituents which are responsible for various pharmacological activities like hepatoprotective, insecticidal, larvicidal, analgesic, microbial infections (Gopi *et al.* 2015). *Tagetes erecta* flower are rich source of minerals, phytochemicals and antioxidants (Arfin *et al.* 2015). The great antioxidant activity of *Tagetes erecta* flower indicates the potential of the extracts as a source of natural antioxidants or nutraceuticals with possible application to reduce oxidative stress with consequent health benefits (Valyova *et al.* 2012).

Conclusion

From the study it is concluded that the *Tagetes erecta* is a good natural source of secondary metabolites which effectively showed antioxidants as well as anti-inflammatory activities. Fresh leaf and flower extract of

T. erecta possess effective chemical constituents which can have great pharmacological actions in herbal drugs.

Acknowledgement

Author likes to thanks UGC, Rajiv Gandhi National Fellowship for SC students (JRF) for providing financial support to the study.

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