

**PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF
ETHANOLIC EXTRACTS OF *TARGETES ERECTA* AND *ZINGIBER
OFFICINALE***

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Article Received on
29 July 2017,

Revised on 18 August 2017,
Accepted on 07 Sept. 2017

DOI: 10.20959/wjpr201711-9380

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ABSTRACT

Natural drugs play important and vital role in the modern medicine. It is usually used to cure some diseases which may not be treated by conventional medicine. Drugs from the plants are easily available less expensive, safe and efficient and rarely have side effects. The alkaloids, tannins, flavanoids and phenol compounds play a major role in preventing various chronic diseases by a definite physiological action on the human body like anticancer, antimicrobial, antioxidant and anti-diabetic activities. The aim of the present study was to evaluate the phytochemical analysis and antioxidant activity of ethanolic extract of Flowers of *Tagetes Erecta* and rhizome of *Zingiber Officinale*. Phytochemical screening was carried out for ethanolic

extract revealed the presence of various bioactive components include alkaloids, carbohydrates, proteins and saponins. Antioxidant activity was assayed by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, nitroblue tetrazolium (NBT) and ferric reducing power (FRAP). In all the assays, stem extract exhibited stronger antioxidant activity than that of flowers. The present results showed antibacterial and antioxidant activity of the extracts was found to be positively associated with the total phenolic and flavonoid content of the extracts.

KEYWORDS: Antibacterial activity, Antioxidant activity, *Tagetes Erecta*, *Zingiber Officinale*, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ferric reducing power (FRAP).

1. INTRODUCTION

Plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Since ancient times green leafy vegetables have been used as medicine and have been playing a very important role in diet and nutrition. They are the major sources of carbohydrates, proteins, vitamins, minerals, fats, aminoacids and fibres.^[1] The bioactive compounds of plants have a wide range of biological functions including, antimicrobial, antioxidant, anti-inflammatory activities.^[2, 3] Plants have to adapt to the changing environmental conditions for their survival of existence. The oxidative environment presents a range of free radicals including superoxide, hydroxyl radical, nitric oxide and peroxy nitrite, for living organisms to deal with. Many evidences are exists to explain the role of free radicals in the development of various diseases including cancer, neurodegeneration and some inflammatory diseases.^[4, 5] Antioxidants have therefore gained importance for their capacity to neutralize free radicals. In this context, the antibacterial and antioxidant properties of various medicinal plants are being investigated throughout the world because of the toxicological concerns associated with the synthetic antioxidants and preservatives.^[6]

Tagetes erecta commonly known as African Marigold is known for its high therapeutic values belongs to family Asteraceae and the genus *Tagetes* consists of 56 species.^[7] The genus *Tagetes* (derived from Etruscan Tages) originated in North and South America and widely cultivated in other Asian countries like Bhutan, China, Nepal, India etc.^[8] which has a strong historic evidence for its religious and therapeutic value^[9] in the treatment of hiccups, dermatitis, athlete's foot, colitis, wound burns etc.^[10] Decoction of flowers are very effective for cold conjunctivitis, mumps and eye sore.^[11]

Zingiber officinale, commonly known as Ginger, belongs to Zingiberaceae family.^[12] Gengibre, Ancoas are the most frequently used Spanish names of Ginger^[13] but in Pakistan, it is known as "Adrac". The horizontally solid underground rhizome (with elegantly covered skin) of this plant has proved to be one of the most extensively used culinary agent and spice in daily home cooking practice.^[14,15] Despite of its use as flavoring agent, ginger is also appreciated in ayurvedic, tibbe-e-unani allopathic^[16] and aromapathy.^[17] Ginger rhizome can be employed in the form of fresh paste, ginger tea (flavoring), dried powder and preserved slices.^[18] Therefore, the main objective of the present study was to evaluate the

phytochemical analysis and antioxidant activity of ethanolic extract of flowers of *Tagetes Erecta* and rhizome of *Zingiber Officinale*.

2. MATERIALS AND METHODS

2.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid, gallic acid (GA), rutin (RU), nitroblue tetrazolium (NBT), and Folin–Ciocalteu’s reagent, were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Potassium hydroxide, α -naphthol, Mercuric nitrate, Ferric chloride, Lead acetate, Sulphuric acid, Hydrochloric acid, Picric acid, Chloroform and Nitric acid were purchased from SR Scientifics (Tirupathi, India). All other chemical reagents used were of analytical grade.

2.2. Collection of plant material

The fresh flowers of *Tagetes erecta* were collected from the flower garden, near pulivendula India and Ginger rhizomes were collected from a farm near pulivendula, India in March 2017. Collection was performed by pulling plants out of the soil and transferring them into sealable plastic bags.

2.3. Preparation of extracts

Flowers and stems of the plant were collected and dried under shade at room temperature. The plant material was then chopped and ground to fine powder using a mechanical blender. 20gm of powder of flowers of *Tagetes Erecta* and rhizome of *Zingiber Officinale* was taken into conical flask. The phytoconstituents were extracted by adding 100ml of ethanol to the powder. The flask was incubated in orbital shaker for 48 hrs. The extract was filtered through five layers of Muslin cloth. The process was repeated twice. The collected extract was pooled and concentrated by evaporation.^[19] The extract was preserved and stored at 4⁰C in airtight bottles for further study.



Fig 2.1 Stem and flowers of *Zingiber Officinale* and *Tagetes Erecta*



Fig 2.2 Extract powder form of *Tagetes Erecta* and *Zingiber Officinale*

2.4. Phytochemical screening

Phytochemical analysis of flowers of *Tagetes Erecta* and rhizome of *Zingiber Officinale* extracts were carried out to identify various phytoconstituents. The methods for screening were carried out according to the method of^[19,20] with some modifications.

The test for phytochemical screening includes:

a) Test for carbohydrates

Molisch's Test

Extracts were dissolved individually in 5ml distilled water and filtered. Filtrates were treated with two drops of alcoholic α -naphthol solution in a test tube. Add 0.2 ml of concentrated sulfuric acid slowly through the sides of the test tube, a purple to violet colour ring appears at the junction.

b) Test for Proteins and Amino acids

Millons test

Test solution with 2ml of Millons reagent (Mercuric Nitrate in Nitric acid containing traces of nitrous acid), white precipitate appears, which turns red upon gentle heating.

c) Test for fats and fixed oils

Stain test

Press the small quantity of extract between two filter papers. The stain on one filter paper indicates the presence of fixed oils.

Saponification test

Add a few drops of 0.5N of alcoholic potassium hydroxide to small quantities of various extracts along with a drop of phenolphthalein separately and heat on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

d) Test for Alkaloids

Hager's test

Extracts were dissolved individually in dilute hydrochloric acid and filtered. Filtrates were then treated with Hager's reagents (saturated picric acid solution). The presences of alkaloids were confirmed by the formation of yellow colored precipitate.

Wagner's test

Filtrates were treated with wagner's reagent. Formation of brown/reddish brown precipitate indicates the presence of alkaloids.

e) Test for Flavonoids

Lead acetate test

Extract were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Sulphuric acid solution:

Extracts were treated with few drops of sulphuric acid. Formation of orange colour indicates the presence of flavonoids.

f) Test for steroids

2ml of acetic anhydride was added to 0.5g of the extracts of each with 2ml of sulphuric acid. The change of colour from violet to blue or green in samples indicates the presence of steroids.

g) Test for Terpenoids

Salkowski's test

0.2 g of the extract of the whole plant sample was mixed with 2 ml of chloroform and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

h) Test for phenols

Ferric chloride test

Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of phenol.

i) Test for Saponins

Foam test

About 0.2 g of the extract was shaken with 5 ml of distilled water. Formation of frothing shows the presence of saponins.

j) Test for Anthroquinone glycosides

Extracts were treated with 5ml chloroform and shaken for minutes. The extracts were filtered and filtrate was added with equal volume of 10% ammonia solution. A pink violet or red colour was observed for the presence of anthraquinone.

2.5. DPPH assay

The antioxidant activity was determined by DPPH assay as described earlier with some modifications.^[21] From the stock solution different concentrations of extract (100 µg–600 µg/ml) were prepared. 200 µl of each concentration was mixed with 3.8 ml DPPH solution and incubated in the dark at room temperature for 60 min. Absorbance of the mixture was then measured at 517 nm control and Vitamin E was used as a positive. Scavenging ability of the sample to DPPH radical was determined according to the following equation.

$$\% \text{ DPPH scavenging activity} = \frac{(\text{Control absorbance} - \text{Sample absorbance}) \times 100}{\text{Control absorbance}}$$

2.6. Ferric reducing power assay

Ferric reducing/antioxidant power (FRAP) was determined following the method as described earlier.^[22] Briefly, 100 µl of each concentration of the extracts (100–600 µg/ml) was mixed with 2.5 ml of 200 mM phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50 C for 20 min. After this, 2.5 ml of 10% trichloroacetic acid was added and the tubes were centrifuged at 10,000 rpm for 10 min. Five milliliters of the upper layer of the solution was mixed with 5.0 ml of distilled water and 1 ml of 0.1% ferric

chloride and the absorbance of the reaction mixtures was measured at 700 nm. The final results were expressed as mg ascorbic acid equivalent/g of dry weight.

2.7. NBT assay

Superoxide anion scavenging activity was performed as described earlier.^[23] From the stock solution (1 mg/ml) different concentrations of extract (100 µg g–500 µg g/ml) were prepared. The reaction was performed in 50 mM phosphate buffer (pH 7.8) containing extracts of various concentrations (100–600 µg/ml), 1.5 mM riboflavin, 50 mM NBT, 10 mM DL-methionine, and 0.025% v/v Triton X-100. The reaction was initiated by illuminating the reaction mixture and absorbance of formazan was recorded at 560 nm and percentage scavenging activity was described.

2.8. Statistical analysis

Data were expressed as Mean ± SD. Statistical analysis was performed by SPSS 11.5. One-way analysis of variance (ANOVA) was utilized to evaluate differences.

3. RESULTS

3.1. Preliminary phytochemical screening

The results of preliminary phytochemical screening of ethanolic extract of rhizome of *Zingiber Officinale* showed the presence of carbohydrates, alkaloids, Flavanoids, Terpenoids, saponins, Phenols and tannins and ethanolic extract of flowers of *Tagetes Erecta* showed the presence of carbohydrates, alkaloids, Flavanoids and saponins. The pharmacological activities of medicinal plants are due to presence of secondary metabolites such as alkaloids, carbohydrates, proteins and saponins, there is possibility of antioxidant and antimicrobial activity. The phytochemicals play a vital role in preventing renal diseases, cholesterol and carcinomas.^[24] The results of tests are shown in Table 3.1.

Table.3.1. Phytochemical screening of extract flowers of *Tagetes Erecta* and rhizome of *Zingiber Officinale*.

S.No	Phytochemical test	Reagent used (test performed)	Observation	Result of <i>Tagetes Erecta</i>	Result of <i>Zingiber Officinale</i>
1.	Test for Carbohydrates	Molisch's test	Formation of violet ring	++	++
2.	Test for Proteins and amino acids	Millon's test	Formation of red colour	-	-
3.	Test for fats and oil	Saponification test	No formation of soap	-	-
4.	Test for Alkaloids	Wagner's test	Formation of cream precipitate	+++	+++
		Hager's test	Formation of yellow colour	+++	+++
5.	Test for Flavanoids	Lead acetate test	No formation of Yellow precipitate	+	++
		Sulphuric acid test	No formation of orange colour	+	++
6.	Test for Terpenoids	Salkawoski test	No formation of reddish brown	-	+
7.	Test for glycosides	Borntrager's test	No formation of Pink colour	-	-
8.	Test for steroids	Acetic anhydride test	Formation of blue or green colour	-	-
8.	Test for Saponins	Foam test	Formation of foam	+++	+++
9.	Test for Phenols and Tannins	Ferric chloride test	No formation of bluish black colour	-	+

+ sign indicates fairly presence, ++sign indicates moderately presence, +++sign indicates more quantity and – sign indicates absence

3.2. Antioxidant activity

Plants rich in secondary metabolites including phenolics, flavonoids and carotenoids exhibit antioxidant activities which are due to their redox properties and chemical structures. The antioxidant property of the crude extracts was investigated and compared by various biochemical assays like, DPPH and NBT assay. The ethanolic extract of stem of *Zingiber Officinale* demonstrated comparatively stronger antioxidant activity as compared to the flower extract *Tagetes Erecta*. The DPPH scavenging activity was found to be 52%, 80% and 91% at 600 µg/ml for flower extract, stem extract and Vitamin C respectively (Fig. 3.1).

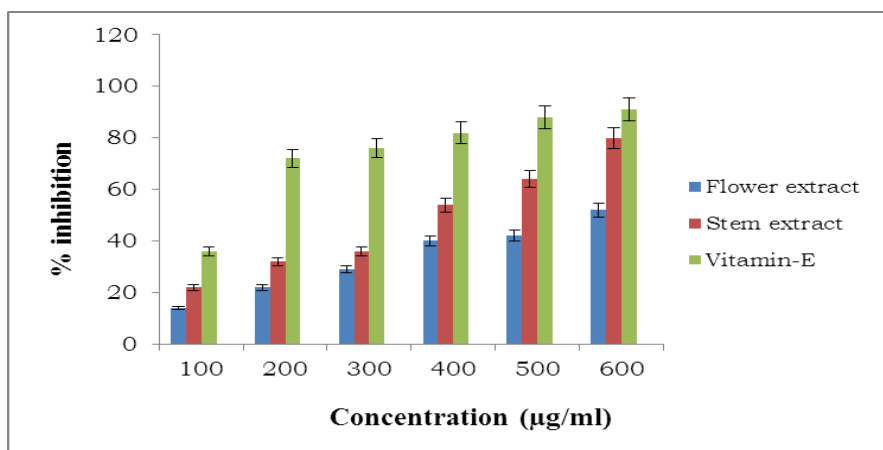


Fig. 3.1: Free radical scavenging activity of ethanolic extracts of flower and stem of *Butea monosperma*. Vitamin E was included as a positive control. Activity was measured by the scavenging of DPPH radicals and each value is expressed as the mean \pm standard deviation.

Superoxide scavenging activity determined by NBT assay was found to be 50%, 72% and 91.7% at 600 $\mu\text{g/ml}$ for flower extract, stem extract and ascorbic acid respectively (Fig. 3.2). Presence of antioxidant substances or reductants in the plant extracts leads to the reduction of Fe^{3+} ferricyanide complex to the ferrous form (Fe^{2+}). We also evaluated the reducing power of the crude extracts and significant changes were observed with the increase in the concentration of the extract (100–500 $\mu\text{g/ml}$). For flower extract absorbance values ranged from 0.28 to 0.62 and for stem extract the values were between 0.32 and 0.64 (Fig 3.3). Ascorbic acid was used as a positive control.

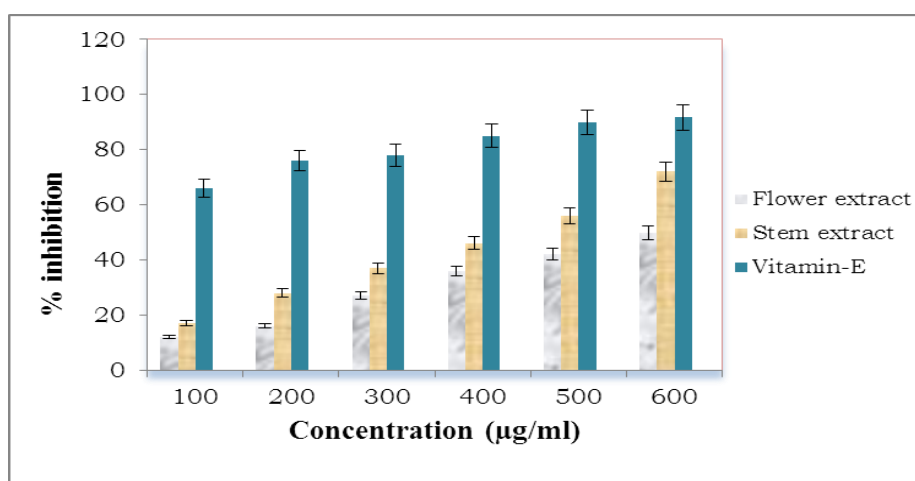


Fig. 3.2 Superoxide scavenging activity of ethanolic extracts of flower and stem of *Butea monosperma*. Vitamin E was included as a positive control. Activity was measured using NBT assay and each value is expressed as the mean \pm standard deviation

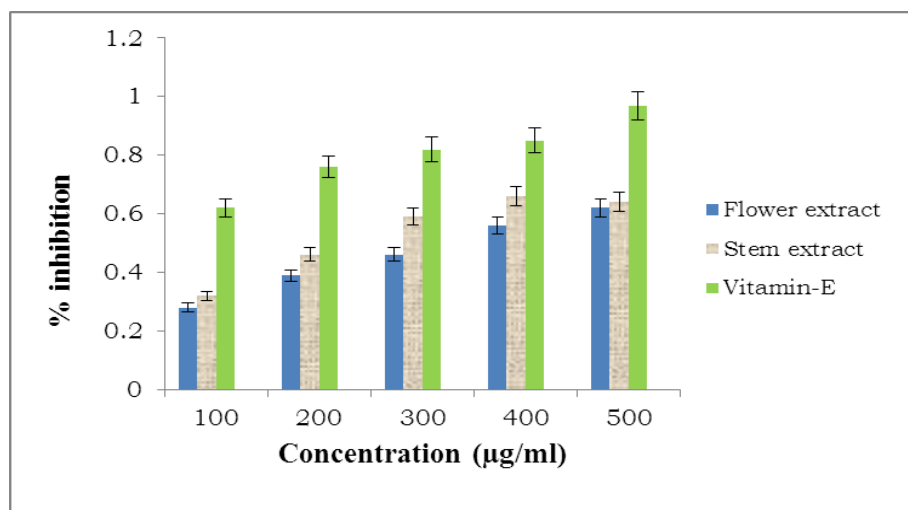


Fig.3.3 Determination of ferrous reducing capacity of ethnolic extracts of flower and stem of *Butea monosperma*. Vitamin E was taken as a positive control. Each value is expressed as the mean \pm standard deviation.

4. DISCUSSION

In present study, we determined the phytochemical screening of ethanolic extract of rhizome of *Zingiber Officinale* showed the presence of carbohydrates, alkaloids, Flavanoids, Terpenoids, saponins, Phenols and tannins and ethanolic extract of flowers of *Tagetes Erecta* showed the presence of carbohydrates, alkaloids, Flavanoids and saponins. Antioxidant activity of these crude extracts may be attributed to the high phenolic and flavonoid content. Phenolic compounds are important plant constituents because of their free radical scavenging ability facilitated by their hydroxyl groups and the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity.^[25] Phenolic compounds are also involved in conferring plants with oxidative stress tolerance. Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals implicated in several diseases.^[26] Flavonoids, on the other hand, suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species, and up-regulate and protect antioxidant defenses.^[27] Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics and flavonoids, are increasingly being used in the food industry for their antioxidative properties and health benefits. In present study, the ethanolic extracts stem of *Zingiber Officinale* showed comparatively higher antioxidant activity than the ethanolic extract flower of *Tagetes Erecta*, which is in accordance with the total phenolic and flavonoid content of the two extracts.

5. CONCLUSION

The results suggest that *Tagetes Erecta* and *Zingiber Officinale* is a potential source of antioxidant molecules. The flowers and stem of the plant can be used as natural antioxidants and preservatives in food and non-food systems. However, further phytochemical analysis is required for the determination of bioactive molecules from the plant that may show a broad spectrum of pharmacological activities.

ACKNOWLEDGMENTS

We acknowledge Dr.K.Aruna,Department of Biotechnology, JNTUACE, Pulivendula for providing necessary chemicals and support for completion of this work.

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