Journal of Inventions in Biomedical and Pharmaceutical Sciences (JIBPS)
ISSN: 2456-818X
Available Online at www.jibps.com

Research article

Evaluation of H_2O_2 radical scavenging activity, phenolic and flavonoid content of the formulated beverage made from *T. erecta L.*

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Key words: *Tagetes erecta*, Activity, H_2O_2 scavenging assay, Flavonoid, Quercetin.

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Vol. 3(3), 01-06, Jul-Sep, 2018.

Abstract

Tagetes erecta, the Mexican marigold, also called Aztec marigold, is a species of the genus *Tagetes* native to Mexico. Despite its being native to the Americas, it is often called African marigold. It is well known for its antimicrobial, antiseptic, wound and ulcer healing, anti-inflammatory, antioxidant and antiviral properties, and it has a long history of being used as an herbal remedy. *T. erecta* produces a variety of substances that possess pharmacological effects and antioxidant activity. The present study aims to analyze the antioxidant property of a beverage made of dried *T. erecta L.* flowers petals , cultivated in West Bengal, India. Radical scavenging potential was determined using H_2O_2 (Hydrogen peroxide) scavenging assay, which showed that with increase in concentration of the sample, the % of inhibition also increased. The total flavonoid content of the residue left after filtering the beverage, made from dried and processed *Tagetes* flower was found to be 530.32 µg/g dry weight, using a standard curve of quercetin. Furthermore, the total phenolic content of the same was estimated, taking a standard curve of Gallic acid, and was calculated to be 78.31µg GAE/ g of dry sample. This study suggests that the flowers of *Tagetes erecta* L. are the possible sources of natural radical scavengers. Thus black tea leaves could be used as natural antioxidants in the beverage, food and pharmaceutical industries that need further wide range *in vivo* studies.

Introduction

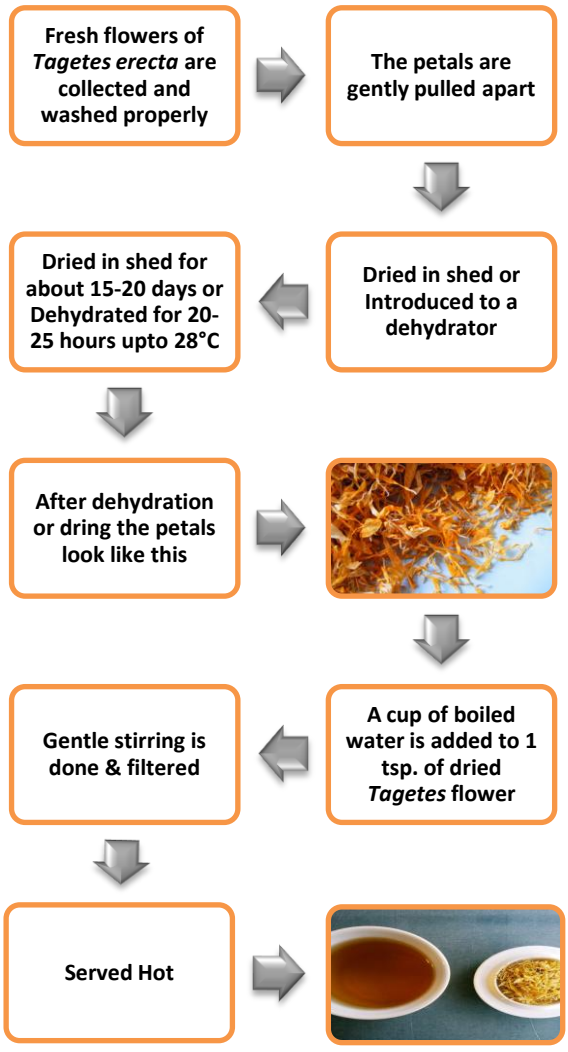
Antioxidants are the substance that inhibits oxidation, especially one used to counteract the deterioration of stored food products. Several decades of dietary research findings suggested that consuming greater amounts of antioxidant-rich foods might help to protect against diseases. Antioxidants are present in phenolic compounds, phenolic acids and their esters, Flavonoids, vitamins, cofactor and minerals, terpenoids carotenoids etc. Because of these reasons, there has been a lot of research on antioxidant based supplements, in recent times. Marigold (*Tagetes erecta* L.) belongs to the family Asteraceae [1-2]. The plant is mainly found in Mexico and now it has been spread to some parts of African continent and to all parts of the world. Apart from its ornamental value, marigold is also well known for its herbal remedy. Different parts of this plant including flowers are used as medicine (using herbal and other remedies based on traditional beliefs) to cure various diseases. Different species of *Tagetes* have been found to possess Anti-bacterial, antimicrobial, anti-inflammatory Blood coagulation property, hepato-protective, wound healing, insecticidal, analgesic activities [3-4]. Tea is one of the most common aromatic beverages which are the most widely consumed drink in the world. It is popular as a recreational drink and also as a medical drink across the globe. Just like Tea, a beverage can also be formulated using the petals of marigold (*Tagetes erecta* L.) flowers, as its pharmacological activity is related to the content of several secondary metabolites, among which the most important compounds are terpenes, essential oils, flavonoids, carotenoids and polyphenols [3-4]. The present study mainly aims at determining the radical scavenging activity, total phenolic and flavonoid contents of *T. erecta* cultivated in West Bengal [5-6].

Requirements for formulation

The requirements for the formulation of the beverage made of the marigold (*Tagetes erecta* L.) flowers are as follows:-

- Freshly collected Marigold (*Tagetes erecta* L.) flowers; as in this case, collection was from West Bengal, India.
- Distilled water
- Hot plate
- Filter paper or mesh-strainer

Preparation procedure [7]



Phytochemical screening of this beverage [8]

Tests	Process & Reagents	Test results	Observation (+/-)
Test for alkaloids			
Dragendroff's Test	KI+ Bismuth nitrate	Orange colored solution	+
Hager's test	Saturated aqueous solution of picric acid	Precipitate	+
Mayer's Test	Potassium mercuric-iodide solution	Yellow precipitation	+
Wagner's Test	I_2 solution	Brown precipitate	+
Test for Carbohydrate			
Fehling's test	Adding Fehling's solution	Red Precipitate	-
Molisch Test	α-napthol solution	Purple Ring	-
Test for fixed oil & fat			
Saponification Test	Adding NaOH solution	Soap formation	-
Test for Glycosides			
Baljet Test	Adding Sodium picrate	Pale Yellow colour	+
Test for Proteins & amino acid			
Biuret test	NaOH solution+ $CuSO_4$	Violet colour	-
Test for Flavonoids			
Shinoda test	Powdered sample + Mg turnings + few drops of Conc. HCl	Scarlet Red or orange Colored solution	+
	Adding $K_2Cr_2O_7$ to the sample	Red or orange precipitation	+
	Sample + $KMnO_4$	Discoloration	+
Test for phenolic			
Ferric chloride Test	3-4 drops of 1% ferric chloride + sample	Bluish or violet colour	+
Lead acetate Test	3 ml of 10% lead acetate solution + sample	Whitish precipitate	+

Physical characteristics of the prepared beverage

- Pale yellow in colour
- Soft aroma of marigold flower
- Not sweet to taste, until added.

Materials & methods of studies

Plant materials

Tagetes erecta L. is not a native plant of India. The flowers of *T. erecta* were collected at full flowering stage from a garden of Texmaco estate in Kolkata, India. The plant along with the flowers were authenticated by Botanical Survey of India, Central National Herbarium, AJC Bose Indian Botanic garden, Howrah, Kolkata (Specimen no:PR03). All other chemicals were of analytical grade and obtained commercially and used as received. The flowers were collected and kept under the knowledge of Guru Nanak Institute of Pharmaceutical Science & Technology. Samples were pure without any additive or preservative materials.

Preparation of the plant materials

The Tagetes flowers were washed properly with water & ethanol, in order to make it free from any sort of pollutants [3, 9]. After washing properly, the *Tagetes erecta* flowers were air dried under shed for about 30 days. After complete drying, the plant material was ground into smaller fragments and passed through sieve number 12. About 2 gms of this dry grounded flower was taken and dipped in 40 ml boiled hot water and kept for 5 minutes. At the 6th minute, the entire process was filtered with the help of Whatman No. 1 filter paper. The residue left on the Whatman No. 1 filter paper is again air dried for 3 days. The dried residue is taken as test sample.

Most flavonoids unlike phenolic compounds are unstable when interacts with high temperature. The objective of taking the filtered residue as the test sample is for the estimation of flavonoid content of the dried Tagetes flowers after being dipped into hot boiled water for 5 mins. This would enable us to find out how much flavonoid and phenolics are present in the consumed hot beverage.

Determination of total phenolic content

The total phenolic content for the given sample was determined by Folin Ciocalteu's method. For this 1 ml of the aliquot is taken along with standard gallic acid in test tubes. To this 5 ml of distilled water was added along with 0.5 ml of Folin Ciocalteu's reagent. The content of the test tubes were mixed properly and shaken thereafter. The mixture was kept for 5 min and to that 1.5 ml of 20% Na_2CO_3 was added the volume made up to 10 ml with the addition of distilled water. The whole mixture is allowed to incubate for 2 hrs at room temperature. An intense blue colour was developed and the absorbance was measured at 750 nm using UV-Visible Jasco V-630 instrument. The extracts were performed in triplicates. The blank was performed using reagent blank with solvent. Gallic acid was used as standard. The calibration curve can be plotted by using standard gallic acid. The reading for total phenolic contents of the herbal formulation were expressed as μg of gallic acid equivalent weight (GAE)/ g of dry mass [9-10].

Determination of total flavonoid content

The total flavonoid content of the test sample was determined by using of a spectrophotometric method [11-12].

The air-dried test sample (25 mg) was ground in a mortar with 10 ml 80 % methanol. The homogenous mixture obtained was allowed to stand for 20 min. at room temperature, followed by filtration through Whatman No. 1 filter paper. 1ml of aliquots and 1ml standard quercetin solution (100, 200, 400, 600, 800, 1000 μg/ml) was positioned into test tubes and 4ml of distilled water and 0.3 ml of 5 % sodium nitrite solution was added into each. After 5 minutes, 0.3 ml of 10 % aluminum chloride was added. At 6th minute, 2 ml of 1 M sodium hydroxide was added. Finally, volume was making up to 10 ml with distilled water and mix well. Orange yellowish color was developed. The absorbance was analysed by UV-Visible Jasco V-630 at 510 nm spectrophotometer. The blank reading was taken by distilled water. Quercetin was used as standard. Sampling was done in triplicates. The calibration curve was plotted (Figure 3) using standard quercetin. The data of total flavonoids of polyherbal formulation were expressed as μg of quercetin equivalents/g of dry mass.

Hydrogen peroxide scavenging (H_2O_2) assay

Human beings are subjected to exposure to H_2O_2 by the environment at 0.28 mg/kg/day mostly by leaf crops [12-13]. Hydrogen peroxide usually enters into the human body through inhalation of vapor or mist and through eye or skin contact [17]. H_2O_2 is decomposed into oxygen and water and this which can

produce hydroxyl radicals (OH^-) and initiates lipid peroxidation which causes DNA damage in the body [14].

The ability of plant extracts to scavenge hydrogen peroxide can be estimated according to the method of Ruch *et al.* [6]. A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM pH 7.4). Hydrogen peroxide's concentration is determined by absorption at 230 nm using a spectrophotometer [19]. The extract (20–60 µg/mL) in distilled water is added to hydrogen peroxide and the absorbance was noted at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide [14]. The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\% \text{ scavenged } (H_2O_2) = [(A_i - A_t)/A_i]*100$$

Where A_i is the absorbance of control and A_t is the absorbance of test.

Results and discussions

Determination of total phenolic content

The total phenolic content of the test sample was referred by Folin Ciocalteu's method using gallic acid as standard. The blue colour exhibited has a maximum absorption in the region of 750 nm and proportional to the total quantity of phenolic compounds which was present. The gallic acid solution of concentration (10-100 ppm) confirmed to Beer's Law at 750 nm with a regression co-efficient (R^2) =0.987. The plot has a slope (m) = 0.0106 and intercept = 0.0542. The equation of standard curve is y = 0.011x + 0.050 (Figure 1).

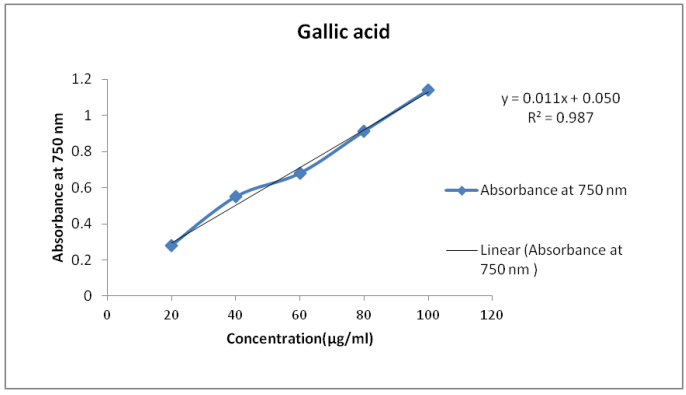


Figure 1. Total phenolic content for standard gallic acid.
R^2 values represented mean data set of n=3.

Phenolics are well established to show antioxidant activity and contribute to human health. The content of phenolics was evaluated and expressed in GAE as micrograms per gram of dry sample (µg GAE/g) [14-15]. The total phenolic content of the test sample was found to be 78.31µg GAE/ g of dry sample and a calibration curve of Gallic acid were used (Figure 2).

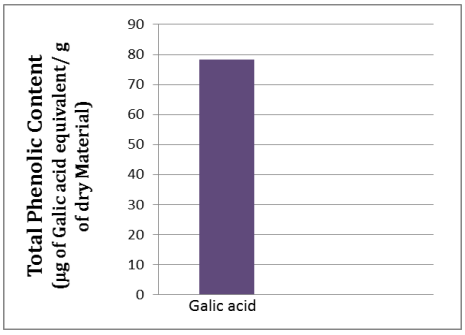


Figure 2. Total flavonoid content of the Test Sample in µg GAE /g.

Determination of total flavonoid content

This test was performed by referring to a journal which has used Aluminium chloride colorimetric assay using quercetin as standard [15]. Aluminium chloride forms acid stable complexes with the C-4 keto groups and with the C-3 or C-5 hydroxide group of flavones and flavonols. It also forms complexes with ortho dihydroxide groups in A/B rings of flavonoids [3, 8]. The quercetin solution of concentration (100-1000 ppm) conformed to Beer's Law at 510nm with a regression co-efficient (R^2) = 0.9994. The plot has a slope (m) = 0.0005 and intercept = 0.029. The equation of standard curve is y = 0.0005x + 0.029 (Figure 3).

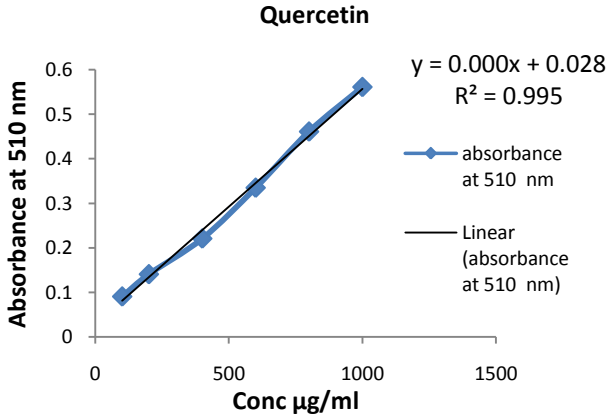


Figure 3. Total phenolic content for standard quercetin.
R^2 values represented mean data set of n=3.

The content of total flavonoids was measured via UV-Visible spectrometer by the method of aluminium chloride colorimetric assay. The flavonoid content of the test Samples was expressed as Quercetin equivalents in µg/g dry weight (530.32 µg/g dry weight) and a calibration curve of Quercetin were used (Figure 4).

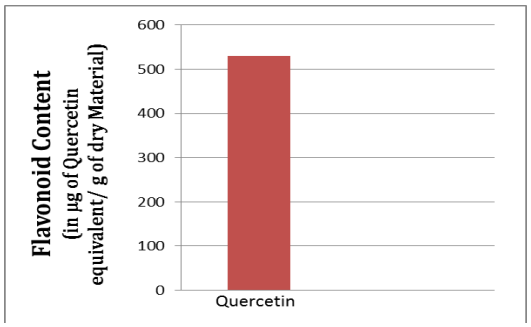


Figure 4. Total flavonoid content of the Test Sample in µg/g.

Hydrogen peroxide scavenging (H_2O_2) assay

H_2O_2 is highly important because of its ability to penetrate into biological membranes [16]. H_2O_2 itself is not very reactive, but it is toxic to cell because it may produce hydroxyl radicals in the cells. H_2O_2 scavenging activity by extracts can be linked to their phenolics, which are capable of donating electrons to H_2O_2, and neutralizes it to water. The results showed the extracts had potent H_2O_2 scavenging activity which might be due to the antioxidant compounds. As the antioxidant components present in the extracts are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O [17]. The antioxidant activity as per the hydrogen peroxide scavenging assay is compared with the ascorbic acid and shown in figure 5 and 6.

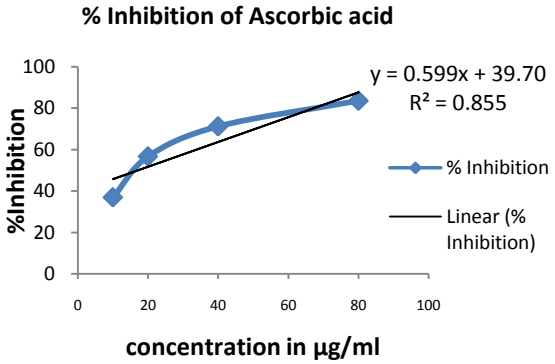


Figure 5. H_2O_2 scavenging activity of the ascorbic acid.

Here, The regression co-efficient (R^2) = 0.855. The plot has a slope (m) = 0.599 and intercept = 39.70.

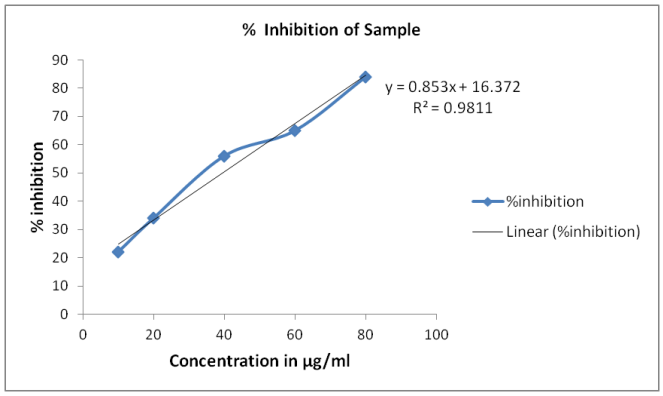


Figure 6. H_2O_2 scavenging activity of the Sample.

According to this antioxidant study (H_2O_2 scavenging assay), the data collected by the analysis shows that, when the concentration of the sample is low then minimum % inhibition of oxidation is observed. As the concentration of the sample increases, % inhibition of oxidation also increases.

Conclusion

Tagetes erecta or Marigold flower beverage in this research exhibited different degrees of antioxidant activity. This study indicates that *Tagetes erecta* is one of the most effective flowers in terms of antioxidant properties and a beverage made from it can serve as natural sources to the free radical scavengers and antioxidant agents. *Tagetes erecta* flowers can be considered as promising sources of natural antioxidants and as possible preventative agents of some common human health disorders [18]. The total phenolic content of the residue of the beverage made from the *Tagetes erecta* flower was found 78.31µg GAE/ g of dry sample as per Gallic acid standards and flavonoid content of the same was found 530.32 µg/g of dry weight as per quercetin standards. However, detailed studies on the role of individual phytochemicals involved in the antioxidant activity of this plant are required for its use as functional food, beverage and in the pharmaceutical industry.

Acknowledgment

The authors are grateful to Guru Nanak Institute of Pharmaceutical Science and Technology for providing provisions and facilities to complete the research work.

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