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PROXIMATE ANALYSIS, PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *TAGETES ERECTA* LEAVES

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ABSTRACT

Petroleum ether, chloroform, methanol and water extract of Bangladeshi *Tagetes erecta* leaves investigated for proximate analysis, phytochemical screening and antioxidant activity tests. The moisture content, total ash, acid insoluble ash and water soluble ash value were 14.6%, 15.6%, 6.03% and 6.69% respectively. Water extract exhibited higher extractive value among four extracts. Phytochemical evaluation of methanol and water extracts confirmed the presence of alkaloids, flavonoids, reducing sugars, saponins, glycosides and proteins & amino acids. For the evaluation of antioxidant activity, three complementary test systems namely reducing power assay, total antioxidant capacity, reduction of ferric ion activity determination methods were used. Methanol extract revealed higher reducing power (1.538±0.011), petroleum ether revealed higher total antioxidant capacity (440 AAE/g) and chloroform extracts revealed higher ferric ion chelating activity than other extracts at 500 µg/ml concentration.

KEYWORDS: *Tagetes erecta*, proximate analysis, phytochemicals, Antioxidant.

INTRODUCTION

Phytochemistry is the name given to the study of the chemistry of plants. Like animals, plants produce a wide variety of chemical compounds, called metabolites, as part of their normal life processes. These compounds perform different functions. For example, some enable plants to store energy in the form of sugar, whilst others are protective against disease or predators. Plants and plant-based medicines are the basis of many of the modern pharmaceuticals we use today for our various ailments.[1] The medicinal value of plants lies

in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids and phenolic compounds. Free radicals are chemical species, which contain one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Free radicals are generated as part of the body's normal metabolic process and play a dual role in our body as both deleterious and beneficial species. Excess production of reactive oxygen species (ROS) and/or a decrease in antioxidant levels may lead to the tissue damage and different diseases. Antioxidant plays a major role in protecting our body from disease by reducing the oxidative damage to cellular component caused by ROS. Recent investigations suggest that the plant origin antioxidants with free radical scavenging properties may have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effect; while relatively plant based medicines confer fewer side effects than the synthetic drug in some instances.[2] *Tagetes erecta* is one of the medicinal plants of Bangladesh locally known as Gendaful (Marigold). It is a stout, branching herb, native to Mexico and other warmer parts of America and neutralized elsewhere in the tropics and subtropics including India and Bangladesh.[3] These are rapid growing annual flowering plant with heights ranging from dwarfs of 6-8 inch to medium and taller and erect growing plants height from 10 inch to 3 ft, bearing large pompon-like double flowers up to 5 inch across and have a shorter flowering period from mid-summer to frost.[4] *Tagetes erecta* has been documented for various medicinal properties such as antioxidant[5], antibacterial[6], antonociceptive and diuretic activities[7], anti- inflammatory[8], wound healing properties[9], mosquitocidal potency[10] etc. The aim and objective of the current study was to investigate the chemical groups present, evaluate the possible antioxidant activity of petroleum ether, chloroform, methanol and aqueous extracts of Bangladeshi *Tagetes erecta* leaves to justify its use in traditional treatments.

MATERIALS AND METHODS

Collection and Preparation of Sample

The leaves of *Tagetes erecta* was collected from Noakhali Science and Technology University, Bangladesh. The leaves were washed properly and then air dried for several days. The leaves were then ground into coarse powder using high capacity grinding machine.

Proximate Analysis

Proximate analysis of a substance constitutes different classes of nutrients present in the samples such as moisture content, total ash, acid insoluble ash, water soluble ash values and extractive values.

Determination of moisture content: Accurately weighed 5 g of powdered of *Tagetes erecta* leaves were taken in a crucible. It was kept in a hot air oven at 105 – 110 ˚C, until free from moisture. The percentage of moisture content was then calculated with reference to the air-dried sample.

Determination of total ash value: Accurately weighed 5 g of powdered *Tagetes erecta* leaves were taken in a dried silica crucible. It was incinerated at 450 ˚C temperature, until free from carbon and then cooled. The weight of ash was taken and the percentage of it was calculated with reference to the air-dried sample.

Determination of acid insoluble ash value: The total ash obtained was boiled for 5 minutes with 25 ml of 2 N HCl, filtered and the insoluble matter was collected on ashless filter paper. Then, it was washed with hot water, ignited in silica crucible for 15 minutes at temperature not exceeding 450 ˚C, cooled and weighed the obtained residue. The percentage of acid insoluble ash was calculated with reference to the air-dried sample.

Determination of water soluble ash value: The total ash obtained was boiled with 25 ml of water for few minutes, filtered and the insoluble matter was collected on ashless filter paper. Then, it was washed with hot water, ignited in silica crucible for 15 minutes at temperature not exceeding 450 ˚C, cooled and weighed the obtained residue. The difference in weight represents the water soluble ash. Finally, the percentage of water soluble ash was calculated with reference to the air-dried sample.

Determination of petroleum ether, chloroform, methanol and water-soluble extractive value: 20 g of air dried, coarsely powdered *Tagetes erecta* leaves were macerated with 100 ml of petroleum ether in a closed flask for 24 hrs, shaking frequently during the first 6 hrs and was allowed to stand for 18 hrs. Then it was filtered rapidly and precautions were taken against loss of petroleum ether. 25 ml of the filtrate was evaporated to dryness in a Petri dish, dried at 105 °C and weighed. The percentages of petroleum ether soluble extracts were

calculated with reference to the air dried sample. The procedure followed as above using chloroform, methanol and water instead of petroleum ether.

Sequential Extraction

The method is based on the extraction of active constituents present in the drug using various solvents ranging from non-polar to polar. The solvents used are petroleum ether, chloroform, methanol and water. The successive solvent extraction procedure was adopted for the preparation of various extracts of *Tagetes erecta* leaves. The materials were subjected to successive extraction with solvents in their ascending order of polarity (non-polar to polar). In this process, the substance which is soluble in a solvent with particular range of polarity was extracted in the solvent and remaining marc further extracted with next solvent. The powder (200 g) was extracted sequentially for 8 hours in petroleum ether, chloroform and methanol using a Soxhlet apparatus. After methanol extraction, the remaining dried marc was extracted with water to get water extract. For the preparation of aqueous extract, the above dried marc was macerated for 3 days with distilled water and the residue was removed by filtration and filtrate was concentrated to obtain aqueous extract. All the extracts were concentrated with a rotary evaporator and dried using oven dryer at 35-40 ˚C. Dried extracts were stored for further use.

Preliminary Phytochemical Screening

Phytochemical screening of different extracts for the presence of alkaloids, flavonoids, reducing sugars, saponins, glycosides, steroids, tannins and proteins & amino acids were carried out.

Test for alkaloids: Solvent free extracts, 50 mg was stirred with few ml of dilute hydrochloric acid and filtered separately. The filtrate was tested carefully with various alkaloid reagents as follows.

***Mayer's test*:** To 1 ml filtrate of the extract, 0.5 ml of Mayer's reagent (potassium mercuric iodide) was added by the side of the test tube. A white or creamy or yellow colour precipitate was formed and that was indicated as the presence of alkaloids.

***Wagner's test*:** To 1 ml filtrate of the extract, 0.5 ml of Wagner's reagent was added by the side of the test tube. Reddish brown precipitate was formed and that was indicated as the presence of alkaloids.

***Hager's test*:** To 1 ml filtrate of the extract, 0.5 ml of saturated picric acid solution (Hager's reagent) was added by the side of the test tube. Yellowish precipitate was formed and that was indicated as the presence of alkaloids.

***Dragendroff's test*:** To 1 ml filtrate of the extract, 0.5 ml of Dragendroff's reagent (bismuth nitrate) was added by the side of the test tube. A prominent yellow or orange brown precipitate was formed and that was indicated as the presence of alkaloids.

Test for flavonoids: The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

Test for reducing sugars: Extracts were dissolved individually in 5 ml of distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

***Benedict's test*:** Filtrate was treated with Benedict's reagent and heated on water bath. Formation of an orange red precipitate indicated the presence of reducing sugars.

***Fehling's test*:** Filtrate was hydrolyzed with dilute hydrochloric acid, neutralized with alkali and heated with Fehling's A and B solutions. A red precipitate was formed which indicated the presence of carbohydrates.

Test for saponins: The extracts were diluted with 20 ml of distilled water separately and further shaken for 15 min in a graduated cylinder. A layer of foam measuring about 1 cm was formed which indicated the presence of saponins.

Test for glycosides: Extracts were hydrolyzed with dilute HCl and then subjected to test for glycosides. Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammonical layer showed the presence of glycosides.

Test for steroids: The extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride boiled and cooled concentrated sulphuric acid was added through the sides of the test tube. The formation of brown coloured ring at the junction of two liquids confirmed the presence of steroids.

Test for tannins: 50 mg of extracts were boiled in 20 ml of distilled water separately and filtered. The filtrates were used to test the presence of tannins.

Ferric chloride test: 1 ml of 5% Ferric chloride solution was added in 1 ml of extracts solution. Greenish black precipitate was formed and indicated the presence of tannins.

***Potassium dichromate test*:** 2 ml solution of the extract was taken in a test tube. Then 0.5 ml of 10% potassium dichromate solution was added. A yellow precipitate was formed indicates the presence of tannins.

Test for proteins & amino acids: The extracts were treated with 4-5 drops of concentrated nitric acid. Formation of yellow colour indicated the presence of proteins.

Antioxidant Activity

In order to investigate the antioxidant properties of the extracts, reducing power assay, total antioxidant capacity and reduction of ferric ions by *ortho*-phenanthroline colour method were performed.

Reducing power assay: The reducing power was based on Fe (III) to Fe (II) transformation in the presence of the solvent extracts.[11] The Fe (II) can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Various concentrations of the sample (2 ml) were mixed with 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of potassium ferricyanide (10 mg/ml). The mixture was incubated at 50 °C for 20 min followed by addition of 2 ml of trichloroacetic acid (100 mg/l). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution. A volume of 2 ml from each of the mixture earlier mentioned was mixed with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) fresh ferric chloride. After 10 min reaction, the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates a higher reducing power.

Total antioxidant capacity: The total antioxidant capacity of the extracts was determined by phosphomolybdate method using ascorbic acid as a standard.[12] An aliquot of 0.1 ml of sample solution was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 35°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. A typical blank contained

1 ml of the reagent solution and the appropriate volume of the solvent and incubated under the same conditions.

Reduction of ferric ions by *ortho*-phenanthroline colour method: A reaction mixture containing 1 ml *ortho*-phenanthroline (5 mg in 10 ml methanol), 2 ml ferric chloride 0.2 mM (3.24 mg in 100 ml distilled water) and 2 ml of various concentrations of the extracts was incubated at ambient temperature for 10 min, then the absorbance was measured at 510 nm. Ascorbic acid and gallic acid were used as reference standards.

RESULTS AND DISCUSSION

Proximate Analysis

The powdered leaves of *Tagetes erecta* was subjected to evaluate its moisture content, total ash, acid insoluble ash, water-soluble ash value and petroleum ether, chloroform, methanol & water-soluble extractive value. The air dried sample contains 14.6% moisture. The low moisture content of the leaf would hinder the growth of microorganism and storage life would be high.[13] The total ash (15.6%) indicates that the leaf is comparatively rich in mineral elements. Acid insoluble and water soluble ash values were found 6.03% and 6.69% respectively. The extractive values for petroleum ether, chloroform, methanol and water were 0.9%, 2.18%, 7.1% and 10.7% respectively (Table 1).

Table 1: Moisture content, ash value and extractive value of *Tagetes erecta*.

Moisture content	Ash value			Extractive value			
	Total ash	Acid insoluble ash	Water soluble ash	Petroleum ether	Chloroform	Methanol	Water
14.6%	15.6%	6.03%	6.69%	0.9%	2.18%	7.1%	10.7%

Preliminary Phytochemical Screening

All the four extracts obtained from successive solvent extraction were subjected to qualitative chemical evaluation to detect the chemical constituents present in them. Petroleum ether extract revealed only flavonoids. Chloroform extracts revealed flavonoids and proteins & amino acids. Methanol extracts showed the presence of alkaloids, flavonoids, reducing sugars, saponins and proteins & amino acids. Water extract showed the presence of alkaloids, flavonoids, reducing sugars, saponins, tannins and proteins & amino acids (Table 2).

Table 2: Qualitative chemical analysis of different solvent extracts of *Tagetes erecta*.

Tests	Extracts			
	Petroleum ether	Chloroform	Methanol	Water
Alkaloids	-	-	+	+
Flavonoids	+	+	+	+
Reducing sugars	-	-	+	+
Saponins	-	-	+	+
Glycosides	-	-	-	-
Steroids	-	-	-	-
Tannins	-	-	-	+
Proteins & amino acids	-	+	+	+

Antioxidant Activity

Reducing power assay: Figure 1 shows the dose response curves for the reducing powers of all extracts (31.25-500 µg/ml) from *Tagetes erecta* leaves. Methanol extract showed (1.538±0.011) higher reducing power than other extracts but lower than standard BHT (2.005±0.001) at 500 µg/ml concentration. In reducing power assay, the yellow colour of the test solution changes to green depending on the reducing power of the test specimen. The presence of the reductants in the solution causes the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by absorbance measurement at 700 nm. Previous reports suggested that the reducing properties have been shown to exert antioxidant action by donating of a hydrogen atom to break the free radical chain. Increasing absorbance at 700 nm indicates an increase in reducing ability. The antioxidants present in the extracts of *Tagetes erecta* caused their reduction of Fe^{3+}/ferricyanide complex to the ferrous form and thus proved the reducing power.

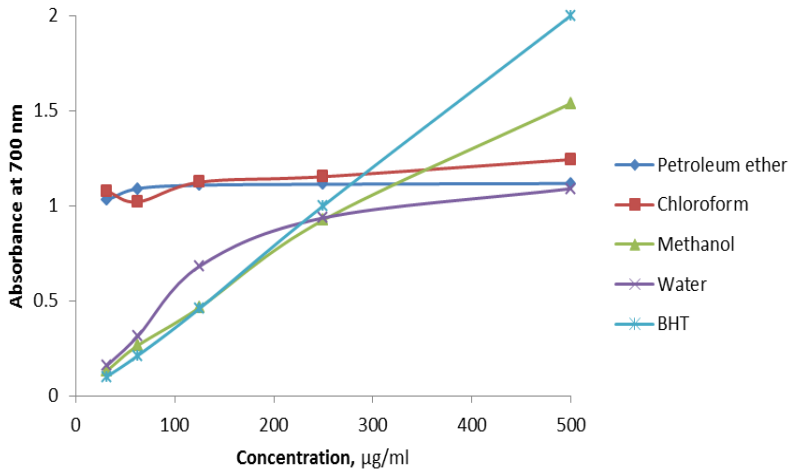

Figure 1: Reducing power assay of different extracts of *Tagetes erecta* with standard.

Total antioxidant capacity: The phosphomolybdate method is quantitative, since the total antioxidant capacity is expressed as ascorbic acid equivalents. The absorbance at 695 nm versus concentration of different extracts and standard curves were shown in figure 2. The antioxidant capacity of various solvent extracts (500 µg/ml) of *Tagetes erecta* leaves were found to decrease in this order: petroleum ether (440 AAE/g) > chloroform (383 AAE/g) > methanol (269 AAE/g) > water (158 AAE/g). The antioxidant capacity of the extracts were measured spectrophotometrically through phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 695 nm. The present study demonstrated that petroleum ether extract exhibited the highest antioxidant capacity for phosphomolybdate reduction. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants.[14, 15]

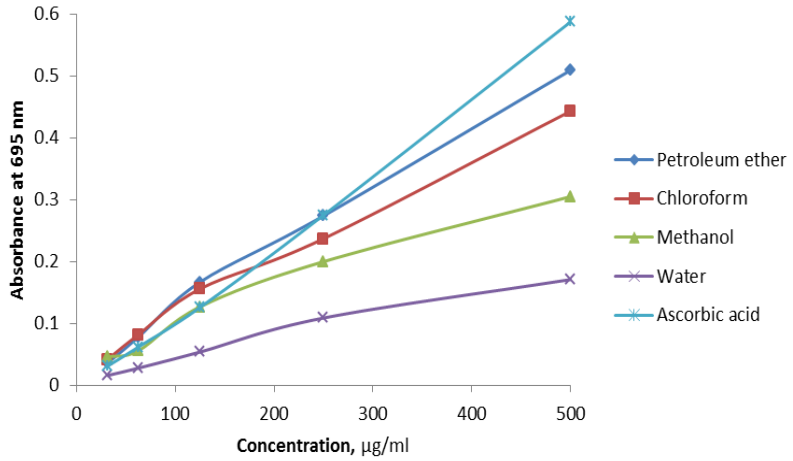

Figure 2: Total antioxidant capacity of different extracts of *Tagetes erecta* leaves with standard.

Reduction of ferric ions by *ortho*-phenanthroline colour method: *Ortho*-substituted phenolic compounds may exert pro-oxidant effects by interacting with iron. *O*-phenanthroline quantitatively forms complexes with ferric ion which get disrupted in the presence of chelating agents. The extracts interfered with the formation of ferrous-*o*-phenanthroline complex, thereby suggesting that the extract has metal chelating activity. The chloroform extract and petroleum ether extracts showed higher chelating activity than standard ascorbic acid and gallic acid. The methanol and water extracts also contributed fairly higher chelating activity (figure 3).

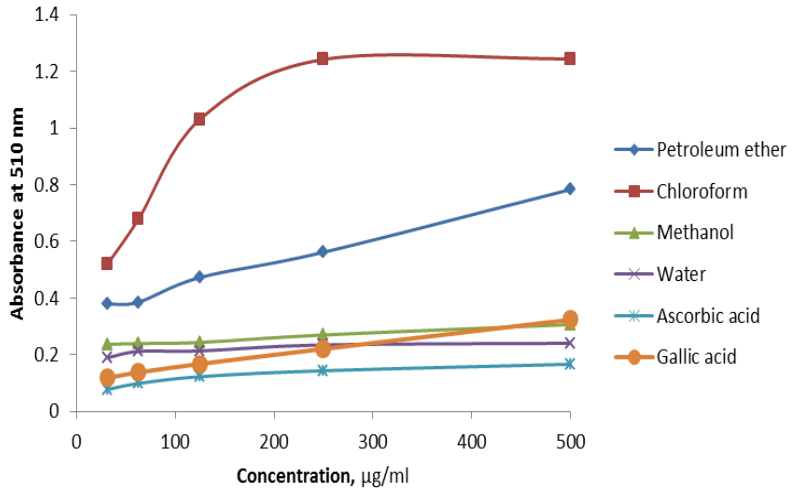

Figure 3: Reduction of ferric ions of different extracts of *Tagetes erecta* leaves by *ortho*-phenanthroline colour method.

CONCLUSION

It can be concluded that *Tagetes erecta* leaves contains different phytochemical constituents and antioxidant activity so the plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of chemically interesting and biologically important drug candidates.

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REFERENCES

1. Poonam, K. and Singh, G.S. Ethnobotanical study of medicinal plants used by the Taungya community in Terai.
2. Cotton, C.M. Ethnobotany: Principle and application. New York: John Wiley and Sons., 1996; 399.
3. Nikkon, F., Habib, M.R., Saud, Z.A., Karim, M.R., Roy, A.K., Zaman, S. Toxicological Evalution of chloroform fraction of flower of *Tagetes erecta* L. on rats. *International Journal of Drug Development and Research.*, 2009; 1: 67-71.

4. Vijay, K.P., Laxman, B.C., Balasaheb, S.R., Yuvraj, N.R., Janardhan, P.M. Pharmacognostic, physicochemical & phytochemical investigation of *Tagetes erecta* Flower (Asteraceae). *Journal of Biological & Scientific Opinion.*, 2013; 1(1): 21-24.
5. Gong, Y., Liu, X., Hao, W., Xu, H-G., Yuan, F., Gao, Y.-X. Invastigation into the antioxidant activity and chemical composition of alcoholic extracts from defatted marigold (*Tagetes erecta* L.) Residue. *Fitoterapia*, 2012; 83: 930.
6. Hashim, H., Kamali, E.L. and Mohammad. Antibacterial and phytochemical screening of ethanolic extracts obtained from selected Sudanese medicinal plants. *J Biol Sci*, 2010; 2(2): 143-146.
7. Hossain, M.A., and Biva, I.J. Antinociceptive and Diuretic Activities of *Tagetes erecta* Linn. *World Journal of Pharmaceutical Science.*, 2014; 2(1): 133-136.
8. Vaghasiya Y *et al*. Antimicrobial and Antiinflammatory screening of four Indian medicinal plants. *Lat Am J Pharma.*, 2011; 30(4): 661-666.
9. Chatterjee, S., Prakash, T., Kotrsha, D., Rao, R.N., Goli, D. Comparative Efficacy of *Tagetes erecta* and *Centella asiatica* extracts on Wound Healing in Albino Rats. *Scientific Research*, 2011; 2: 138–142.
10. Nikkon, F., Habib, M.R., Saud, Z. and Karim, M.R. *Tagetes erecta* Linn and its mosqquitocidal potency against *Culex quinuefasciatus*. *Asian Pacific Journal of tropical Biomedicine*, 2001; 1(3): 186-188.
11. Adeyeye, E.I. and Ayejuyo, O.O. Chemical composition of *cola accuminata* and *Grarcinakola* seed grown in Nigeria. *Int J Food Sci Nutri.*, 1994; 45: 223-230.
12. Sharififar, F., Dehghn-Nudeh, G. and Mirtajaldini, M. Major flavonoids with antioxidant activity from *Teucrium polium L. Food Chem.*, 2009; 112: 885–888.
13. Khan, R.A., Khan, M.R. and Sahreen, S. Assessment of flavonoids contents and *in vitro* antioxidant activity of *Launaea procumbens*. *Chem Central J.*, 2012; 6: 43.
14. Sharififar, F., Dehghn-Nudeh, G., Mirtajaldini, M. Major flavonoids with antioxidant activity from *Teucrium polium L. Food Chem.*, 2009; 112: 885–888.
15. Khan, R.A., Khan, M.R., Sahreen, S. Assessment of flavonoids contents and *in vitro* antioxidant activity of *Launaea procumbens*. *Chem Central J.*, 2012; 6: 43.