



**Research Paper**

**PROXIMATE ANALYSIS, PHYTOCHEMICAL SCREENING AND  
ANTIOXIDANT ACTIVITY OF *Tagetes erecta* FLOWER GROWING IN  
COASTAL AREA OF BANGLADESH**

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**Abstract**

Petroleum ether, chloroform, methanol and water extract of Bangladeshi marigold (*Tagetes erecta*) flower, a plant belonging to the family Asteraceae, investigated for proximate analysis, phytochemical screening and antioxidant activity tests. The total moisture content, ash value, acid insoluble ash value and water soluble ash value were 11.42%, 7.98%, 4.085% and 3.18% respectively. Water extract exhibited higher extractive value among four extracts. Phytochemical evaluation of methanolic and water extracts confirmed the presence of alkaloids, carbohydrates, saponins, tannins, proteins, flavonoids. For the evaluation of antioxidant activity, two complementary test systems namely reducing power assay and total antioxidant capacity determination method were used. In reducing power assay test, water extract revealed maximum antioxidant presence having 1.945 at 500 µg/ml, while total antioxidant capacity was quite high in chloroform and methanol extracts (567 and 493 ascorbic acid equivalent per gram of dry extract).

Key words: *Tagetes erecta*, proximate analysis, phytochemicals, Antioxidant.

**INTRODUCTION**

Herbal therapy has come of age and today, medicinal plants play a significant role in human health care globally [1]. In USA about 25% of all prescriptions dispensed in public pharmacies contain drugs extracted from higher plants and about 64% of the total global population remains dependent on traditional medicine for their healthcare needs [2]. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects [3]. As one of developing countries, rural people of Bangladesh also rely on traditional medicine for their primary health care need. *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 naturalized elsewhere in the tropics and subtropics including India and Bangladesh [4]. 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 has a shorter flowering period from mid-summer to frost [5]. *Tagetes erecta* has been documented for various medicinal properties such as antioxidant [6], antibacterial [7], antinociceptive and diuretic activities [8], anti-inflammatory [9], wound healing properties [10], mosquitocidal

potency [11] etc. So far, no systematic chemical investigation has been carried out on the flower part of the *Tagetes erecta*, growing in coastal region of Bangladesh. So the aim and objective of the current study was to investigate the chemical constituents in *Tagetes erecta* flower by photochemical screening and to find out antioxidant activity for exploring the possibility of developing new drug candidates for the treatment of various diseases.

## MATERIALS AND METHODS

### Collection and Preparation of Sample

For this present investigation fresh flower plant was collected from Noakhali Science and Technology University, Sonapur, Noakhali, coastal region of Bangladesh. The collected flowers were separated from undesirable materials or plants or plant parts. They were dried in shade for 20 days. The plant parts were ground into a coarse powder with the help of a suitable grinder.

### Proximate Analysis

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

**Determination of Moisture Content:** Accurately weighed 5 g of powdered of *Tagetes erecta* flowers 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* flowers 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* flower was 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* flower. 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, phenolic compounds & tannins, proteins & amino acids were carried out.

**Test for alkaloids:** Solvent free extract, 50 mg is stirred with few ml of dilute hydrochloric acid and filtered. The filtrate is 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 color 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 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:** The extracts were treated with 4-5 drops of conc. nitric acid. Formation of yellow color indicated the presence of proteins.

**Test for glycosides:** Extracts were hydrolyzed with dil. 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.

#### **Antioxidant Activity**

In order to investigate the antioxidant properties of the examined extracts, reducing power assay and total antioxidant capacity were performed.

**Reducing Power Assay:** The reducing power was based on Fe (III) to Fe (II) transformation in the presence of the solvent extracts [12]. 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 fractions was determined by phosphomolybdate method using ascorbic acid as a standard [13]. 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 765 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. Ascorbic acid was used as standard. The antioxidant capacity was estimated using following formula:

$$\text{Antioxidant effect (\%)} = \frac{\text{Control absorbance (A}_0\text{)} - \text{Sample Absorbance (A)}}{\text{Control absorbance (A}_0\text{)}} \times 100$$

## RESULTS AND DISCUSSION

### Proximate Analysis

The *Tagetes erecta* was subjected to evaluate its total ash value, acid insoluble ash, water-soluble ash, petroleum ether soluble extractive value, chloroform soluble extractive value, methanol soluble extractive value, water-soluble extractive value and moisture content. The air dried sample contains 11.42% moisture. The low moisture content of the leaf would hinder the growth of microorganism and storage life would be high [14]. The ash content of 7.98% indicates that the leaf is comparatively rich in mineral elements. Acid insoluble and water soluble ash values were found 4.08% and 3.18% respectively. The extractive values for petroleum ether, chloroform, methanol and water were 2.92%, 5.11%, 7.8% and 9.74% 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 |
| 11.42%           | 7.98%     | 4.08 %             | 3.18 %            | 2.92%            | 5.11%      | 7.8%     | 9.74% |

### 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 the presence of flavonoids. The chloroform extract shows the presence of flavonoids and proteins. The methanolic and water extract shows the presence of alkaloids, reducing sugars, flavonoids, saponins, tannins (Table 2).

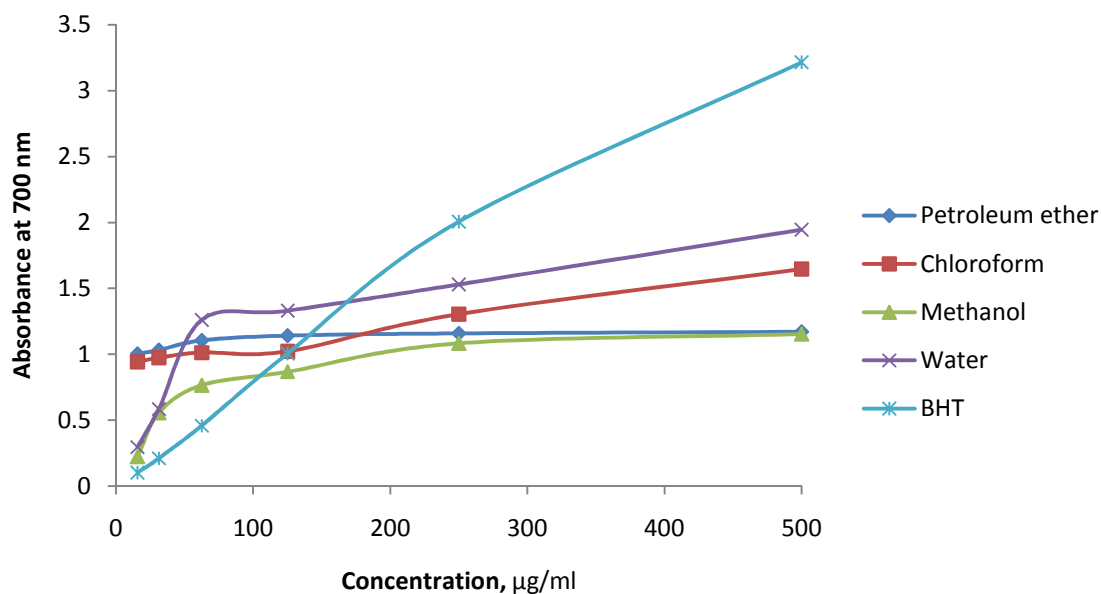
**Table 2: Qualitative chemical analysis of different solvent extracts of *Tagetes erecta*.**

| Tests                       | Extracts        |            |          |       |
|-----------------------------|-----------------|------------|----------|-------|
|                             | Petroleum ether | Chloroform | Methanol | Water |
| <b>Alkaloids</b>            |                 |            |          |       |
| Mayer's Test                | -               | -          | +        | +     |
| Wagner's Test               | -               | -          | +        | +     |
| Hager's Test                | -               | -          | +        | +     |
| Dragendroff's Test          | -               | -          | +        | -     |
| <b>Reducing sugars</b>      |                 |            |          |       |
| Fehling's Test              | -               | -          | +        | +     |
| Benedict's Test             | -               | -          | +        | +     |
| <b>Flavonoids</b>           |                 |            |          |       |
| Lead Acetate Test           | +               | +          | +        | +     |
| <b>Saponins</b>             |                 |            |          |       |
| Froth's Test                | -               | -          | +        | +     |
| <b>Tannins</b>              |                 |            |          |       |
| <i>Ferric Chloride Test</i> | -               | -          | +        | +     |
| Potassium Dichromate Test   | -               | -          | +        | +     |
| <b>Proteins</b>             |                 |            |          |       |
| Xanthoproteic Test          | -               | +          | +        | +     |
| <b>Glycosides</b>           |                 |            |          |       |
| Liebermann-Burchard Test    | -               | -          | -        | -     |

#### Antioxidant activity

**Reducing Power Assay:** Figure 1 shows the dose response curves for the reducing powers of all extracts (15.65-500 µg/ml) from *Tagetes erecta*. Water extract showed (1.945±0.011) more reducing power comparing to other extracts of chloroform, petroleum ether and methanol (1.646±0.016, 1.17±0.013 and 1.153±0.03 respectively) at 500 µg/ml concentration. was BHT was used as standard antioxidant for comparison. All extracts showed weak reducing power than standard BHT (3.215±0.001).

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<sup>3+</sup>/ ferricyanide complex to the ferrous form. Therefore, Fe<sup>2+</sup> 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<sup>3+</sup> / 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 antioxidant capacity of various solvent extracts (500 µg/ml) of *Tagetes erecta* were found to decrease in this order: chloroform (567 AAE/g) > methanol (493 AAE/g) > petroleum ether (336 AAE/g) > water (164 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 765 nm. The present study demonstrated that methanol 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 [15,16].

## CONCLUSION

It can be concluded that *Tagetes erecta* flower 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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