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# *In-vitro* Antioxidant & Anti-inflammatory Potential Evaluations of Methanolic Extract of *Tridax procumbens* (Linn.), a Phytochemical Screening

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**Abstract**

*Tridax procumbens* (Linn.) is a medicinal plant of Bangladesh and this sub continent, which is widely used as folk medicine for the treatment of many diseases. The aim in the present study was to screen the phytochemical profile and pharmacological activities of methanolic extract of *Tridax procumbens* (Linn.) leaves. Because each part of *Tridax procumbens* (Linn.) has different constituents, the pharmacological effects of the plant vary according to the part of the plant evaluated. To investigate pharmacological activities DPPH scavenging assay & HRBC membrane stabilization methods were done for antioxidant and anti-inflammatory potential respectively. The phytochemical analysis of methanolic extract of plant leaves showed that they contained significant presence of flavonoids, phenols, saponins, terpenoids & triterpenes. Alkaloids, glycosides & tannins are also moderately present. Quantitative evaluations show significant presence of phenols than tannin content. The pharmacological studies revealed that the plant extracts may have significant antioxidant effect which is probably mediated by inhibition of DPPH free radical, which is responsible for oxidation. The $IC_{50}$ values by DPPH scavenging assay observed for standard & leaves were 99.31 *µg/ml* & 488.80 *µg/ml* respectively. There is also moderate anti-inflammatory activity. The $IC_{50}$ values for anti-inflammatory activity by standard & leaves were 23.48 *µg/ml* & 833.23 *µg/ml* respectively. These findings suggest that this plant may be a possible source for the development of a new drug.

**Keywords:** *Tridax procumbens*; Phenols; Tannin content; Antioxidant; Anti-inflammatory; $IC_{50}$ values

## Introduction

*Tridax procumbens* (Linn.) (Bengali- Tridhara; Family- Asteraceae) is a medicinal plant which is also spreading annual herb grows up to 20 cm in height. This plant can be found everywhere in fields, meadows, croplands, disturbed areas, lawns, and roadsides of tropical or semi-tropical areas and are known for their medicinal properties among local natives. Plants, which have one or more of its parts having substances that can be used for treatment of diseases, are called medicinal plants [1]. Medicines derived from plants are widely famous due to their safety, easy availability and low cost [2]. Throughout the ages, humans have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavours and fragrances, and medicines [3]. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and probably thousands of years of use. The first records, written on clay tablets in cuneiform, are from Mesopotamia and date from about 2600 BC [4]. Among the substances that were used are oils of *Cedrus* species (cedar) and *Cupressus sempervirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh) and *Papaver somniferum* (poppy juice), all of which are still in use today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation. In ancient Egypt, bishop's weed (*Ammi majus*) was reported to be used to treat vitiligo, a skin condition characterized by a loss of pigmentation [5-6]. More recently, a drug (methoxypsoralen) has been produced from this plant to treat psoriasis and other skin disorders, as well as T-cell lymphoma [6]. Alternative medicine or fringe medicine includes practices claimed to have the healing effects of medicine but which are disproven, unproven, impossible to prove, or are excessively harmful in relation to their effect; and where the scientific consensus is that the therapy does not, or cannot, work because the known laws of nature are violated by its basic claims; or where it is considered so much worse than conventional treatment that it would be unethical to offer as treatment [7]. Alternative therapies or diagnoses are not part of medicine or science based healthcare systems [8]. Alternative medicine consists of a wide variety of practices, products, and therapies ranging from those that are biologically plausible but not well tested, to those with known harmful and toxic effects. Contrary to popular belief, significant expense is paid to test alternative medicine, including over $2.5 billion spent by the United States government [9]. According to the physicians of Unani medicine, three plants *viz.*, *Cassia occidentalis* Linn., *C. sophera* Linn. and *C. sophera* Linn. *var. Purpurea Roxb* are the varieties of 'Kasondi' and are invariably




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conditions. 'Kasondi' is described in Unani literature to be repulsive of morbid humors, resolvent, blood purifier, carminative, purgative, digestive, diaphoretic [10].

## Materials and Methods

### Total Phenolic Content (TPC) determination

In the alkaline condition phenols ionize completely. When Folin-Ciocalteu's reagent is used in this ionized phenolic solution, the reagent will readily oxidize the phenols. Usual colour of Folin-Ciocalteu's reagent is yellow and after the oxidation process the solution becomes blue. The intensity of the colour change is measured in a spectrophotometer at 760 nm. The absorbance value will reflect the total phenolic content of the compound [11].

### Method of sample preparation

The total phenolics of the extracts were determined using the Folin and Ciocalteu reagent, following the method described with slight modifications [12]. The test sample (0.2 mL) was mixed with 0.6 mL of water and 0.2 mL of Folin-Ciocalteu's phenol reagent (1 : 1). After 5 min, 1 mL of saturated sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was made up to 3 mL with distilled water. The reaction was kept in the dark for 30 min and after centrifuging the absorbance of blue colour from different samples was measured at 760 nm. All determinations were carried out in triplicate.

### Total Tannin Content (TTC) determination

Tannins are the complex organic, non nitrogenous derivatives of polyhydroxy benzoic acids which are widely distributed in the plant kingdom. They are present in aerial parts, e.g. leaves, bark, fruits and stem. They probably serve as a protective to the plant during growth and destroyed or deposited as end product of metabolism in some dead tissues of the mature plant. Tannins precipitate and combine with proteins. The protein-tannin complex is resistant to proteolytic enzymes. This property is known as astringent. During healing process of burns, the proteins of the exposed tissues are precipitated producing a mild antiseptic and protective layer under which the new tissues are regenerated. They are used as healing agents in inflammation, leucorrhoea, gonorrhea, burn, piles, and diarrhea and as antidote in the treatment of alkaloidal poisoning [13].

### Method of sample preparation

Fifty micro liters ($\mu l$) of tannins extract for each sample was taken in test tube and volume was made to 1.0 ml with distilled water. Then, 0.5 ml Folin Ciocalteu reagent was added and mixed properly. Then 2.5 ml 20 percent sodium carbonate solution was added and mixed it and kept for 40 minutes at room temperature. Optical density was taken at 725 nm in spectrophotometer and concentration was estimated [14].

## Results and Discussion

### Phytochemical screening

The following tests were done to find the presence of the active chemical constituents such as alkaloids, flavonoids, glycosides, phenols, saponins, tannins, terpenoids and triterpenes is shown in (Tables 1-3). Due to the different chemical compositions present in a *Tridax procumbens* (Linn.) are obviously responsible for its different therapeutic and pharmacological activities. In this study,

**Table 1:** Total Phenolic Content (TPC) of *Tridax procumbens* (Linn.).

| Test sample | Absorbance | TPC (mg of GAE/g) | Average | TPC (mg of GAE/g) ± SEM |
|---|---|---|---|---|
| | 0.202 | 27.308 | | |
| **Leaves** | 0.211 | 27.437 | 27.358 | 27.358 ± 0.36 |
| | 0.209 | 27.115 | | |

Total Phenolic Content (TPC) observed for leaves of *Tridax procumbens* (Linn.) was 27.358 ± 0.36 mg of GAE/g.

**Table 2:** Total Tannin Content (TTC) of *Tridax procumbens* (Linn.).

| Test sample | Absorbance | TTC (mg of TAE/g) | Average | TTC (mg of TAE/g) ± SEM |
|---|---|---|---|---|
| | 0.367 | 1.588 | | |
| **Leaves** | 0.361 | 1.56 | 1.58 | 1.580 ± 0.013 |
| | 0.368 | 1.593 | | |

Total Tannin Content (TTC) observed for leaf of *Tridax procumbens* (Linn.) was 1.580 ± 0.013mg of TAE/g.

**Table 3:** Different chemical compositions resent in plants.

| Secondary metabolites | Name of the test | Results |
|---|---|---|
| Alkaloids | Wagner test | ++ |
| Flavonoids | Specific test | ++ |
| Glycosides | General test | ++ |
| Phenols | Litmus test | +++ |
| Saponins | Froth test | +++ |
| Tannins | Ferric chloride test | ++ |
| Terpenoids | General test | +++ |
| Triterpenes | Salkowski's test | +++ |

**Table 4:** Average absorbance of control.

| | Absorbance | Average |
|---|---|---|
| | 0.452 | |
| **Control** | 0.448 | 0.452 |
| | 0.456 | |

the different constituents of the *Tridax procumbens* (Linn.) which are found should have some relationship with domestic medicinal applications. It should be mentioned here that the presence of these kinds of chemical constituents, it is expected that the selective plant *Tridax procumbens* (Linn.) should have Anti-inflammatory Activity and Anti oxidant activity.

Qualitative evaluations showed significant presence of flavonoids, phenols, saponins, terpenoids, & triterpenes. Alkaloids, glycosides & tannins are also moderately present in the methanolic extract of leaves of *Tridax procumbens* (Linn.).

### Anti-inflammatory activity

Percent inhibition of protein denaturation was calculated as follows [15]:

$$\% \text{ inhibition} = (\text{Control} - \text{Sample}) / (\text{Control})) \times 100$$

The method of HRBC membrane stabilization was chosen to evaluate anti-inflammatory effect. It is already proved that membrane stabilization of RBC is as effective as healing inflammation in provoking delayed hypersensitivity. It revealed that the plant extracts may have moderate anti-inflammatory effect which is probably mediated by HRBC membrane stabilization. The secondary

**Table 5:** Spectroscopic Determination of Anti-inflammatory Activity of Leaves of *Tridax procumbens* (Linn.).

| Concentration (*µg/ml*) | Absorbance | % Inhibition | Average | % Inhibition ± SEM | $IC_{50}$ (*µg/ml*) |
|---|---|---|---|---|---|
| 125 | 0.445 | 1.36 | 1.58 | 1.58 ± 0.7 | |
| | 0.448 | 0.69 | | | |
| | 0.439 | 2.69 | | | |
| 250 | 0.407 | 9.82 | 11.45 | 11.45 ± 0.2 | 833.23 |
| | 0.393 | 12.94 | | | |
| | 0.399 | 11.6 | | | |
| 500 | 0.241 | 46.79 | 46.79 | 46.79 ± 0.7 | |
| | 0.237 | 47.68 | | | |
| | 0.245 | 45.9 | | | |
| 1000 | 0.215 | 52.58 | 53.58 | 53.58 ± 0.50 | |
| | 0.209 | 53.92 | | | |
| | 0.208 | 54.14 | | | |

**Table 6:** Spectroscopic Determination of Anti-inflammatory Activity of Standard Compound (Diclofenac- Na).

| Concentration (*µg/ml*) | Absorbance | % Inhibition | Average | % Inhibition ± SEM | $IC_{50}$ (*µg/ml*) |
|---|---|---|---|---|---|
| 125 | 0.245 | 45.9 | 46.69 | 79.53 ± 0.48 | |
| | 0.241 | 46.79 | | | |
| | 0.239 | 47.24 | | | |
| 250 | 0.163 | 64.16 | 64.1 | 86.01 ± 0.30 | |
| | 0.161 | 64.61 | | | 23.48 |
| | 0.166 | 63.48 | | | |
| 500 | 0.103 | 77.53 | 76.98 | 89.41 ± 0.49 | |
| | 0.109 | 76.19 | | | |
| | 0.105 | 77.08 | | | |
| 1000 | 0.059 | 87.33 | 87.48 | 93.50 ± 0.24 | |
| | 0.056 | 88.27 | | | |
| | 0.061 | 86.9 | | | |

**Table 7:** Comparative study based on $IC_{50}$.

| Test Sample | $IC_{50}$ |
|---|---|
| **Leaves** | 833.23 |
| **Standard** | 23.48 |

metabolites such as phenolic compounds and tannins which were found in preliminary phytochemical screening might be responsible for such type of activity (Tables 4-7).

By analyzing the above data, it revealed that the plant extracts may have moderate anti-inflammatory effect which is probably mediated by HRBC membrane stabilization.

## Anti oxidant activity

The free radical-scavenging activity of extracts was evaluated with the DPPH assay based on the measurement of the reducing ability of antioxidants toward the DPPH radical [16]. By analyzing the above data, it revealed that the plant extracts may have significant antioxidant effect which is probably mediated by inhibition of DPPH free radical, which is responsible for oxidation (Table 8-12).

**Table 8:** Average absorbance of control.

| | Absorbance | Average |
|---|---|---|
| | 0.894 | |
| **Control** | 0.906 | 0.899 |
| | 0.898 | |

**Table 9:** Spectroscopic Determination of Antioxidant Activity of Leaves of *Tridax procumbens* (Linn.).

| Concentration (*µg/ml*) | Absorbance | % SCV | Average | % SCV ± SEM | $IC_{50}$ (*µg/ml*) |
|---|---|---|---|---|---|
| 62.5 | 0.805 | 10.38 | 10.74 | 7.43 ± 0.54 | |
| | 0.809 | 10.06 | | | |
| | 0.797 | 11.76 | | | |
| 125 | 0.676 | 24.27 | 24.69 | 20.23 ± 0.29 | |
| | 0.675 | 24.64 | | | |
| | 0.679 | 25.14 | | | |
| 250 | 0.4266 | 52.56 | 52.89 | 52.89 ± 0.56 | 488.8 |
| | 0.429 | 52.16 | | | |
| | 0.419 | 53.98 | | | |
| 500 | 0.295 | 67.56 | 67.54 | 67.54 ± 0.26 | |
| | 0.289 | 67.99 | | | |
| | 0.297 | 67.1 | | | |
| 1000 | 0.109 | 88.09 | 88.93 | 88.93 ± 0.48 | |
| | 0.096 | 89.66 | | | |
| | 0.1 | 89.04 | | | |
| 2000 | 0.048 | 94.53 | 94.48 | 94.48 ± 0.41 | |
| | 0.056 | 93.79 | | | |
| | 0.049 | 95.09 | | | |

**Table 10:** Spectroscopic Determination of Antioxidant Activity of Standard Compound (L- Ascorbic Acid).

| Concentration (*µg/ml*) | Absorbance | % SCV | Average | % SCV ± SEM | $IC_{50}$ (*µg/ml*) |
|---|---|---|---|---|---|
| 62.5 | 0.348 | 61.79 | 61.92 | 61.92 ± 0.32 | |
| | 0.349 | 61.6 | | | |
| | 0.338 | 62.54 | | | |
| 125 | 0.26 | 71.35 | 70.78 | 70.78 ± 0.35 | |
| | 0.268 | 70.08 | | | |
| | 0.267 | 70.87 | | | |
| 250 | 0.196 | 78.25 | 78.87 | 78.87 ± 0.48 | 99.31 |
| | 0.188 | 79.85 | | | |
| | 0.195 | 78.5 | | | |
| 500 | 0.118 | 86.79 | 87.26 | 87.26 ± 0.28 | |
| | 0.119 | 87.36 | | | |
| | 0.119 | 87.69 | | | |
| 1000 | 0.047 | 94.79 | 94.52 | 94.52 ± 0.17 | |
| | 0.056 | 94.27 | | | |
| | 0.045 | 94.56 | | | |
| 2000 | 0.029 | 97.99 | 96.98 | 96.97 ± 0.55 | |
| | 0.029 | 97.98 | | | |
| | 0.036 | 95.98 | | | |

**Table 11:** Comparative % SCV of DPPH.

| Concentration | Leaves | Standard |
|---|---|---|
| 62.5 *µg/ml* | 10.78 | 61.71 |
| 125 *µg/ml* | 24.67 | 70.77 |
| 250 *µg/ml* | 52.87 | 78.86 |
| 500 *µg/ml* | 67.56 | 87.29 |
| 1000 *µg/ml* | 88.99 | 94.47 |
| 2000 *µg/ml* | 94.47 | 96.97 |

**Table 12:** Comparative study based on $IC_{50}$.

| Test Sample | $IC_{50}$ |
|---|---|
| **Leaves** | 488.8 |
| **Standard** | 99.31 |

By analyzing the above data, it revealed that the plant extracts may have significant antioxidant effect which is probably mediated by inhibition of DPPH free radical, which is responsible for oxidation.

## Conclusion

From my this research work it was found that qualitative evaluations show significant presence of flavonoids, phenols, saponins, terpenoids & triterpenes. Alkaloids, glycosides & tannins are also moderately present. Quantitative evaluations show significant presence of phenols than tannin content. There is an excellent antioxidant activity in the methanolic extract. There is also moderate anti-inflammatory activity in the methanolic extract of leaves. Each part of *Tridax procumbens* (Linn.) has different constituents, the pharmacological effects of the plant vary according to the part of the plant evaluated. Alkaloids, glycosides and tannins are also moderately present. Quantitative evaluations show significant presence of phenols than tannin content. The $IC_{50}$ values by DPPH scavenging assay observed for standard and leaves were 99.31 *µg/ml* and 488.80 *µg/ml* respectively. So, there is an excellent antioxidant activity in the methanolic extract. There is also moderate anti-inflammatory activity in the methanolic extract of leaves. The $IC_{50}$ values for anti-inflammatory activity by standard and plant leaves were 23.48 *µg/ml* and 833.23 *µg/ml* respectively.

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