Int J Pharm Bio Sci 2013 Apr; 4(2): (B) 302 - 311

**Research Article** **Bio chemistry**

**International Journal of Pharma and Bio Sciences** **ISSN 0975-6299**

# ESTIMATION OF PHENOLIC AND FLAVONOIDS CONTENT AND *IN VITRO* ANTIOXIDANT CAPACITY OF *TRIDEX PROCUMBENS* LINN.(ASTERACEAE).

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## ABSTRACT

*Biological source:* - It consists of dried wholes plants of *Tridax procumbens* Linn. belonging to family Asteraceae. *Aim of Study:* The present Study was undertaken to investigate the Total Phenolic Content was estimated by Folin-Ciocaltea Method with an absorbance peak at 765nm using Spectrophotometer and will be expressed as Gallic Acid Equivalents (GAE) in milligram per gram of extract (mg GAE/g extract), Total Flavonoid Content with an absorbance peak at 510nm using spectrophotometer and can be expressed in milligrams of Rutin equivalents (RE) per gram of extract. And most importantly the antioxidant capacity of crude extract of *Tridex procumbens* Linn. using DPPH (*1, 1- Diphenyl-2-picrylhydroxyl)* assay and Hydrogen peroxide ($H_2O_2$) scavenging assay. The antioxidant activities were compared to standard antioxidant ascorbic acid. Ascorbic acid is a naturally occurring antioxidant compound found in medicinal plants, vegetables, fruits and whole grains. Methanolic crude extract of the plant *Tridex procumbens* Linn. showed significant antioxidant activity in DPPH, $H_2O_2$ scavenging methods. The findings of the present study suggested that the plant could be a potential natural source of antioxidants and could have greater importance as therapeutic agents in preventing or slowing oxidative stress related degenerative diseases.

**KEY WORDS:** _***Tridex Procumbens*, Phenolic Content, Flavonoid content, Antioxidant Capacity and $IC_{50}$.**

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# INTRODUCTION

A spreading annual herb grows up to 20 cm in height. Leaves simple, opposite, serrate or dentate, acute, fleshy and pubescent. Flowers in head long stalked and whitish. Seeds are numerous, small with a tuft of silky hairs on one side for wind dispersal. *Tridax procumbens* L. is commonly known as Coat Button or Kansari (in Hindi) or Ghamara (in local language) and belongs to family Asteraceae. It is extensively used in Ayurvedic system of medicine for various ailments and is shown to possess a number of pharmacological activities like hypotensive (Salahdeen et al., 2004), insecticidal, leishmanicidal (Perazasanchez et al., 2007), hair growth promoting (Saraf et al., 1991), wound healing, anti-inflammatory (Margaret et al., 1998), hepatoprotective (Devaki et al., 2005), antiviral (Chien et al., 2001) immunomodulatory (Vyas et al., 2004) and antioxidant activity (Agrawal et al., 2009) due to the presence of phenolics, tannins, saponins, and glycosides. Degenerative disease is caused by free radicals in our body that are able to damage living tissues and cause cell death (Yang et al., 2002). Very quite prominent antioxidant activity has been found in several plant extracts and chemical constituents(Tripathi et al., 1996; Vani et al., 1997; Oseni and Akindahunsi , 2011).Free radicals are produced in normal and/or pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with aging (Halliwell et al., 2003). Awareness of the potential benefits of antioxidant nutrients in health maintenance is growing. Antioxidants are means for the substances or group of the substances that delay or inhibit oxidative damage to a molecule. This defense system is having many modes of classification such as based on their mechanism of action (chain breaking, preventive) (Racek et. al., 2005, Khaw et al., 2001). Investigations into the health maintaining properties of plants have resulted in the identification of a wide array of bioactive compounds in plants that include flavonoids, phenolics, limonoids, carotenoids, coumarins, phytosterols, etc. Based on recent research, several compounds from fruits and vegetables were found to possess anticarcinogenic and antioxidant activities (Okonogi et al., 2007). The literature survey reveals that *Tridax procumbens* L. plant posses good antioxidant activity. (Nia et al., 2003). The present study has been under- taken to estimate the Flavonoid, Phenolic content of *Tridex procumbens* L. Further the most importantly, free radical inhibition activity that is the *in vitro* antioxidant capacity of alcoholic (Methanol) extract of *Tridex Procumbens* L., using the most adequate, simple and least error, DPPH (*1,1-diphenyl-2-picrylhydroxyl)* Assay.

# MATERIALS AND METHODS

### *Preparation of Plant Material*

The plant material was collected from the local village “Shamsabad” of Bhopal, Madhya Pardesh. The identification and authentication of plant specimen was *Tridex procumbens,* done by an expert botanist Dr. Sail Bala Sanghi, Professor Botany M.L.B. College Bhopal, M.P. and the whole Plant material after weighing was dried under shade at room temperature.

### *Preparation of Extract*

The dried plant material was grinded to a coarse powdered form, and was kept for defatting with Petroleum ether and extracted exhaustively with methanol at 45 degree temperature, in a soxhlet extractor. The extract was concentrated in Vaccum Rotary

Evaporator and residue was dried in petridish till the crystalline form was available.

### *Chemical and reagents*

Methanol, Folin-Ciocalteu reagent, $NaHCO_3$, gallic acid, aluminum chloride hexahydrate solution ($AlCl_3$, $6H_2O$), rutin, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) , ascorbic acid. were purchased from Sigma-Aldrich (GmbH, Sternheim, Germany). $H_2O_2$, Phosphate Buffer Saline (Nacl, KCl, $Na_2HPO_4$, $KH_2PO_4$, dd $H_2O$).

### *Determination of total Phenolics Content*

The concentration of phenolics in plant extracts was determined using spectrophotometric method (Singleton *et al*., 1999). Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared**,** containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were there after incubated at room temperature in dark for 45 min. The absorbance was determined using spectrophotometer at λmax = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and a dilution series of gallic acid of concentration 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml was prepared and calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in the extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

### *Determination of total flavonoids content*

The content of flavonoids in the examined plant extracts was determined using spectrophotometric method (Quettier *et al*., 2000). The sample contained 1 ml of methanol solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% AlCl3 solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λmax = 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin and a dilution series of rutin of concentration 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml was prepared and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in the extracts was expressed in terms of rutin equivalent (mg of RU/g of extract).

### *Evaluation of antioxidant activity*

### *DPPH Assay*

***Principal:*** The Capacity of biological reagents to scavenge the DPPH radical, can be expressed as its magnitude of antioxidant ability. The DPPH alcohol solution is deep purple in colour with an absorbance peak of 517 nm. Which appears with the presence of the radical scavenger in the reactive system and when an odd electron of the Nitrogen in the DPPH is paired.

***Method:*** The ability of the plant extract to scavenge DPPH free radicals was assessed by the standard method (Tekao et al., 1994), adopted with suitable modifications (Kumarasamy *et al*., 2007). The stock solution of extracts were prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain a concentrations of 25, 50, 75, 100 and 125 µg/ml. Diluted solutions (1 ml each) were mixed with 1 ml of methanol solution of DPPH in concentration of 1 mg/ml. After 30 min incubation in darkness at room temperature (23 $^oC$), the absorbance was recorded at 517 nm. Control sample contains all the reagents except the extract. Percentage inhibition was calculated using equation 1, whilst $IC_{50}$ values were estimated from the percentage inhibition versus concentration plot,

using a non-linear regression algorithm. The data were presented as mean values ± standard deviation (n = 3).

Equation 1

$$\% \, inhibition = \frac{A \, control - A \, Sample}{A \, control} X \, 100$$

Where "A" stands for Absorbance.

***Hydrogen peroxide scavenging assay***

***Principal:*** $H_2O_2$ itself is not very reactive but it can sometimes be toxic to cell, because of it may give rise to hydroxyl radical in the cells.

***Method:*** *A Solution of $H_2O_2$ (20mM / 40mM):* The 3% $H_2O_2$ is 0.88M. Prepare a 20mM solution (by adding 22.7 µL of the provided $H_2O_2$ to 977 µL of Buffer) in Phosphate buffer saline (PH = 7.4). Various concentrations of 1 ml of the extracts or standards in methanol were added to 2ml of $H_2O_2$ solution in PBS. The absorbance will be measured at 230 nm after 10 minutes of incubation against a blank solution that contained extracts in PBS without hydrogen peroxide (Czochra and Widensk , 2002).The percentage scavenging activity was calculated using equation 2.

Equation 2

$$\% \, Scavenged \, [H2O2] = \frac{A \, control - A \, Sample}{A \, control} X \, 100$$

Where "A" stands for Absorbance.

***Preparation of Phosphate Buffer Saline***

| **1X PBS** | **10X PBS** |
|---|---|
| Reagents Needed | Reagents Needed |
| 8 g Nacl | 80 g Nacl |
| 0.2 g KCl | 2 g KCl |
| 1.44 g $Na_2HPO_4$ | 14.4 g$Na_2HPO_4$ |
| 0.24 g $KH_2PO_4$ | 2.4 g$KH_2PO_4$ |
| 800 ml dd$H_2O$ | 800 ml dd$H_2O$ |

Dissolve all dry reagents together in 800 ml of dd$H_2O$. Adjust the PH to 7.4 using Hcl. Add dd$H_2O$ to a final volume of 1L. Sterilize by autoclaving.

***STATISTICAL ANALYSIS***

All experimental measurements were carried out in triplicate and are expressed as average of three analyses ± standard deviation.

## RESULTS AND DISCUSSION

Table 1 and Table 2 present the results of Concentration verses Absorbance of Gallic acid and Rutin respectively, while Figure 1 and Figure 2 presents the standard calibration plots for the determination of Phenols and Flavonoids respectively.

***Total Phenolics***

The total phenolic contents in the examined plant extracts using the Folin Ciocalteu's reagent is expressed in terms of gallic acid equivalent ( the standard curve equation : y = 0.005x – 0.065, $R^2$ = 0.976). The values obtained for the concentration of total phenols are expressed as mg of GA/g of extract (Table 3). The total phenolic contents in the examined plant extract were 109.2667± 0.41663 mg of GA/g of extract. The total phenolic content in plant extracts depends on the type of extract, i.e. the polarity of solvent used in extraction. The high solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction (Mohsen and Ammar, 2008; Zhou and YU, 2004)

***Total Flavonoids***

The concentration of flavonoids in plant extract of *Tridex procumbens* Linn.was determined using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of rutin equivalent (the standard curve equation: y = 0.001x + 0.118, $R^2$ = 0.985), mg of RU/g of extract (Table 4). The total Flavonoid content in the examined plant extract was 40 ± 2.645751 mg of RU/g of extract. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Min and Chun-Zhao, 2005).

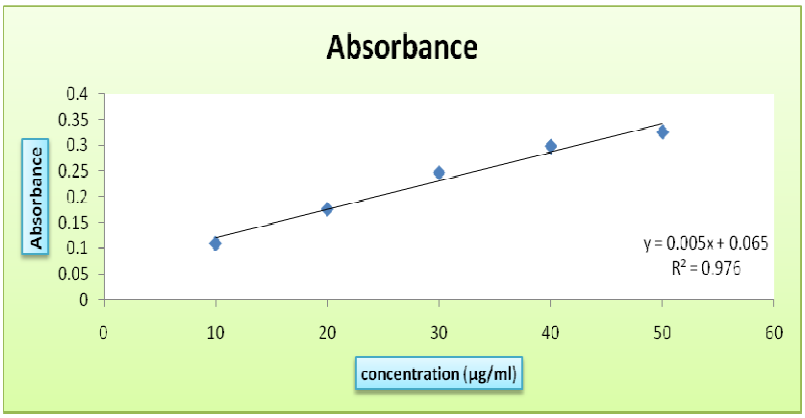


**Figure1**
***Showing the Calibration plot for the determination of Phenols.***

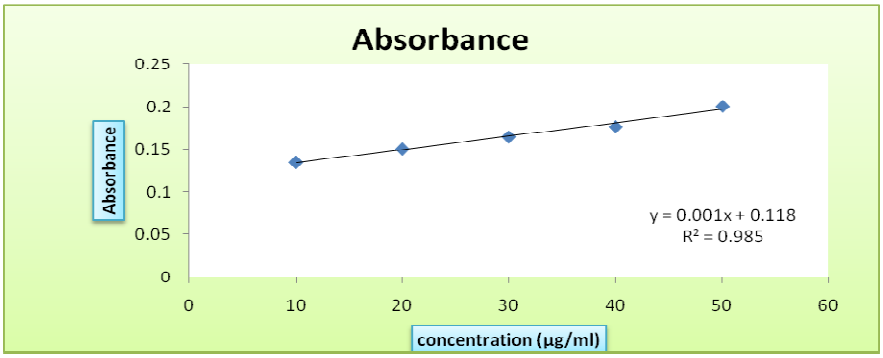


**Figure 2**
***Showing the Calibration Plot for the determination of Flavonoids.***

**Table 1**
***Concentration Verses Absorbance of Gallic acid***

| S. No. | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1 | 10 | 0.1098 |
| 2 | 20 | 0.1763 |
| 3 | 30 | 0.2468 |
| 4 | 40 | 0.2981 |
| 5 | 50 | 0.3258 |

**Table 2**
***Concentration Verses Absorbance of Rutin***

| S. No. | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1 | 10 | 0.135 |
| 2 | 20 | 0.151 |
| 3 | 30 | 0.165 |
| 4 | 40 | 0.177 |
| 5 | 50 | 0.201 |

**Table 3**
***Total Phenolic content in extract expressed in mg/g equivalent to Gallic Acid of Extract.***

| S.No. | Absorbance of Extract | Conc. Of Extract | Total Phenolic Content mg/g equiv. to Gallic Acid |
|---|---|---|---|
| 1 | 0.612 | 1mg/ml | 109.4 |
| 2 | 0.609 | 1mg/ml | 108.8 |
| 3 | 0.613 | 1mg/ml | 109.6 |
| MEAN±SD | | | 109.2667 ± 0.41663 |

**Table 4**
***Total Flavonoid content in extract espressed in mg/g equivalent to Rutin of extract.***

| S.No. | Absorbance of Extract | Conc. Of Extract | Total Flavonoid Content mg/g equiv. to Rutin |
|---|---|---|---|
| 1 | 0.161 | 1mg/ml | 43 |
| 2 | 0.157 | 1mg/ml | 39 |
| 3 | 0.156 | 1mg/ml | 38 |
| MEAN±SD | | | 40 ± 2.645751 |

***Antioxidant activity***

***DPPH Assay***

The antioxidant activity of the methanol crude extract was determined using a methanol solution of DPPH solution, as DPPH is very stable free radical. There is no certainty of any side reaction like metal ion chelation and enzyme inhibition as found in *in vitro* generated free radicals such as hydroxyl radical and superoxide anion. A deep purple colour of freshly prepared DPPH fades when antioxidant molecules quench DPPH free radicals ( i.e. by providing hydrogen atom or by electron donation, conceivably via a free radical attack on th DPPH molecule) and convert them into colorless/ bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm band (Amarowicz, et al., 2003). The antioxidant activity of plant extract is expressed in terms of % inhibition and IC50 Value (µg/ml).The IC50 value is defined as the concentration that causes a decrease in the initial amount of DPPH radicals by 50% (Huang et al.,2005). It is the concentration where the active crude extract will exhibit 50% of antioxidant activity (Chiang et al., 2003) and crude extracts exhibit 50% of inhibition at concentration less than 20 µg $mL^{-1}$. These concentrations are considered positive for antioxidant activity (Geran et al., 1972). The methanolic extract of *Tridex procumbens* Linn. have shown significant activity comparable to the activity of Ascorbic acid as standard, as shown in Table. 5 and Figure 3

**Table 5**
***Evaluation for antioxidant capacity of methanolic extract of Tridex procumbens Linn. (DPPH Assay).***

| | Concentration (µg/ml) and % inhibition | | | | | |
|---|---|---|---|---|---|---|
| Conc. | 25 | 50 | 75 | 100 | 125 | $IC_{50}$ (µg/ml) |
| ASA | 30.75 ± 0.38 | 37.49 ± 0.51 | 49.32 ± 0.39 | 53.07 ± 0.32 | 65.37 ± 0.75 | 83.30 |
| ME | 2.01 ± 0.95 | 20.2 ± 1.15 | 27.23 ± 0.44 | 39.83 ± 1.68 | 52.79 ± 0.13 | 119.72 |

***Conc. Stands for Concentration in µg/ml, ASA stands for Ascorbic acid and ME stands for Methanolic extract of Tridex procumbens Linn.***

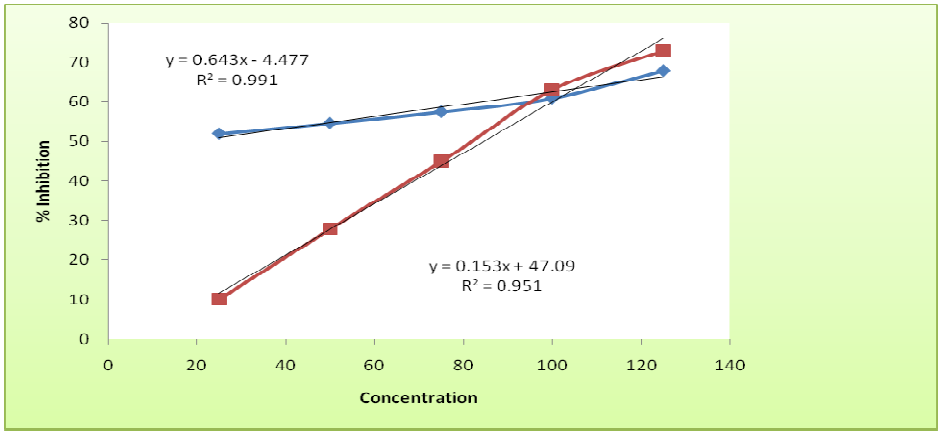


**Figure 3**
***Showing the % Inhibition of methanolic extract of Tridex procumbens using DPPH assay.***

***$H_2O_2$ Scavenging Assay***

$H_2O_2$ is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membrane rapidly. Once inside the cell, $H_2O_2$ can probably react with Fe+2 and possibly Cu+2 to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. Scavenging of $H_2O_2$ may attributed to their phenolics which can donate electrons to $H_2O_2$, thus neutralizing it to water ( Nabavi et al., 2008b; Ebrahimzadeh et al., 2009d). The ability of the extract to effectively scavenge hydrogen peroxide, determined according to the method of Ruch et al., 1989, where they are compared with that of ascorbic acid. The extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner. $IC_{50}$ for scavenging of $H_2O_2$ was 56.96 µg/ml of methanolic extract of *Tridex procumbens* Linn. while that of ascorbic acid was 19.01 µg/ml. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing $H_2O_2$ is very important throughout food systems (Nabavi *et al.,* 2008a). The results are evaluated in table 6 and Figure 4.

**Table 6**
***Evaluation for antioxidant capacity of methanolic extract of Tridex procumbens Linn. ($H_2O_2$ Scavenging Assay)***

| | Concentration (µg/ml) and % inhibition | | | | | |
|---|---|---|---|---|---|---|
| Conc. | 25 | 50 | 75 | 100 | 125 | $IC_{50}$ (µg/ml) |
| ASA | 51.94 ± 1.40 | 54.64 ± 0.92 | 57.48 ± 1.07 | 60.92 ± 1.21 | 67.96 ± 1.31 | 19.01 |
| ME | 10.11 ± 0.91 | 27.9 ± 1.08 | 44.96 ± 0.81 | 63.03 ± 1.35 | 73.01 ± 0.99 | 56.96 |

***Conc. Stands for Concentration, ASA stands for Ascorbic acid, ME stands for Methanol Extract of Tridex procumbens Linn.***

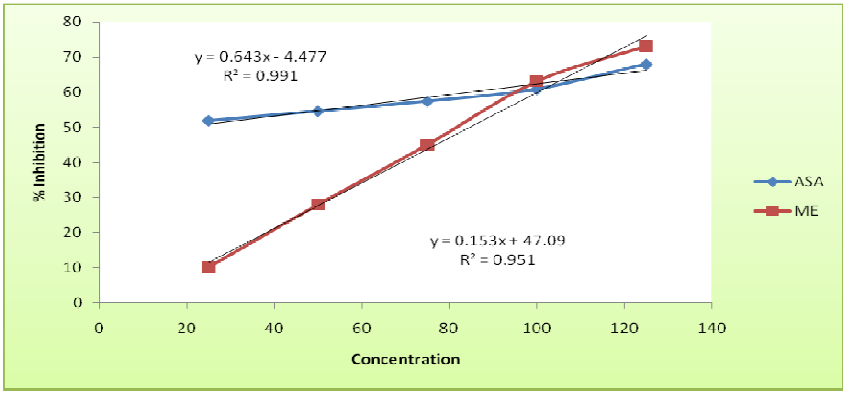


**Figure 4**
***Showing the $H_2O_2$ Scavenging activity of methanolic extract of Tridex procumbens Linn.***

## CONCLUSION

The total phenolic content of the methanolic extract of *Tridex procumbens* Linn. was 109.2667 ± 0.41663 and that of Flavonoid content was 40 ± 2.645751. Further the methanolic extract of *Tridex procumbens* Linn. showed hydrogen peroxide scavenging and powerful antioxidant activities as compared to the standard ascorbic acid. Hence the results of this study on a methanolic extract of *Tridex procumbens* Linn. shows that it can be used as easily accessible source of antioxidant. Keeping in view its high antioxidant property, this plant can also be used alone or in combination in the form of different herbal formulations to protect the body from the deleterious effects of free radicals or in pharmaceuticals.

## AKNOWLEDGEMENT

I am very thankful to M.S. Karchule, Amit Nayak, Pinnacle Biomedical Research Institute, Shymla Hills Bhopal M.P. for providing the necessary laboratory facilities for the conduct of this research work and also thankful to all the staff members of PBRI who assisted me from time to time at the prime time of my research at PBRI.

***OBJECTIVES***

i) To determine the amount of total phenolic and total flavonoids content exists in the *Tridex procumbens* Linn.
ii) To determine the antioxidant activity of methanolic crude extract of *Tridex procumbens* Linn.

***SCOPE OF THE STUDY***

i) Only 70 % methanolic crude extract of *Tridex procumbens* is used for experiment.
ii) Total Phenolic Content (TPC) is determined by using Folin-Ciocalteu method.
iii) Total flavonoid content (TF) is determined by using the Catechin method.
iv) Each sample of TPC (Total Phenolic Content) and TF (Total Flavonoids) is determined by UV-visible spectrometer.
v) Antioxidant activity is determined using DPPH assay and $H_2O_2$ assay.

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