UK Journal of Pharmaceutical and Biosciences
Available at www.ukjpb.com
ISSN: 2347-9442

Evaluation of Phytochemical and Antioxidant Activity of *Tridax procumbens* Extract

Dr Pooja Singh[1*], Dr Kirti Jain[2], Dr Swati Khare[3], Dr Padma Shrivastav[1]

[1]Govt. PG College, BHEL, Bhopal (M.P.)-462016, India
[2]Govt. Science and Commerce College, Benazir, Bhopal (M.P.)-462001, India
[3]Maharani Laxmibai, Govt Girls PG Autonomous College, Bhopal (M.P.)-462016, India

Article Information
Received 21 August 2017
Received in revised form 23 Dec 2017
Accepted 24 December 2017

Keywords:
Tridax procumbens
Phytochemical
Antioxidant Activity
Flavonoids

Corresponding Author:
E-mail : puji.singh86@yahoo.in
Mob.: xxxxxxxxxx

Abstract

Oxidative stress and impaired antioxidant system have been implicated in the pathophysiology of diverse disease states. The polyphenol and flavonoids are used for the treatment of various diseases triggered by oxidative stress. *Tridax procumbens* have been used as indigenous medicine for a variety of ailments. In the present study, it was planned to investigate the phytochemical and *in vitro* antioxidant activity of the ethanol, methanol and aqueous extracts of *Tridax procumbens* leaves. The ethanol, methanol and aqueous extracts of *Tridax procumbens* leaves were prepared and performed its phytochemical analysis. The *in vitro* antioxidant activity namely DPPH, total polyphenol content, total flavonol content and reducing power assay were performed. The qualitative chemical test exhibited the presence of alkaloids, polyphenol, tannins, flavonoids, carbohydrates, saponin and glycoside in ethanol, methanol and aqueous extracts of *Tridax procumbens*. The findings of *in vitro* antioxidant activity demonstrated that ethanol extracts expressed higher antioxidant activity compared to methanol and aqueous extract. These results are an indication of antioxidant potential of the extracts and may be responsible for some of the therapeutic uses of *Tridax procumbens*.

1 Introduction

The imbalance between the production of reactive oxidizing agents and the ability of a biological system to counteract the reactive intermediates results in oxidative stress. The reactive oxidizing agents are formed during normal physiological processes or under stress conditions that can damage biological system[1].

The reactive oxygen species that are constantly generated in the human body cause oxidative stress. The ratio of reactive oxygen species may be increased by the factors such as drugs, chlorinated compounds, deficiency of natural antioxidants, alcohol, stress and unhealthy food. Despite naturally occurring antioxidant systems in the human body, reactive oxygen species cause lipid, protein, and DNA oxidation. These damages at the molecular level may influence the etiology of diseases, such as cancer, kidney failure, atherosclerosis, diabetes, hepatotoxicity, neurodegenerative disorders, and aging-related diseases[2]. It has been demonstrated that antioxidant substances may be defensive against above mentioned diseases.

Many people consume antioxidants as a defense against oxidative stress. The synthetic antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene, and tertiary butyl hydroquinone are associated with the toxic due to presence of higher amount of preservatives. Hence, ascertaining potential natural antioxidant sources can be a useful alternative to ensure better health[3]. One of the special sources of antioxidants is plant-based natural phenolic compounds. Phenolic compounds are found to be one of the most important groups of plant secondary metabolites, due to their great participation in morphological development, physiological processes and reproduction[4]. The phenolic compound imparts antiallergenic, antiatherogenic, anticancer, immunomodulator, hepatoprotective, anti-inflammatory, antimicrobial and antithrombotic agents[3]. The plant containing higher amount of

phenolic component as antioxidant constituents has always directed researchers to search for novel medications to develop healthy life for humans. In addition, some medicinal plants are still obscured within the plant which needs to be scientifically evaluated.

Tridax procumbens belonging to family Asteraceae, and is a common medicinal herb used by ethno-medical practitioners. It is best known as a widespread weed and pest plant[5]. Traditionally, it is used for the treatment of bronchial catarrh, dysentery, malaria, stomachache, diarrhea, high blood pressure and to check hemorrhage from cuts, bruises, and wounds and to prevent falling of hair[6]. The leaves extract has been scientifically documented as analgesic, anti-anemic, anticoagulant, hepatoprotective, anticancer, hypocholesterolemic, antifungal, and insect repellent[7].

The alkaloids, carotenoids, flavonoids (catechins and flavones), saponins, and tannins has been investigated from the leaves of *Tridax procumbens*. Dexamethasone luteolin, glucoluteolin, β-sitosterol quercetin, β-sitosterol-3-O-β-Dxylopyranoside, and flavonoid procumbenetin have been isolated from leaves[10].

The current study planned to investigate the phytochemical and antioxidant activity of the leaves of *Tridax procumbens*. Further to discusses the potentials of the use of the leaves as a functional food, or medicines.

2 Materials and Methods

2.1 Plant material

The leaves of *Tridax procumbens* were collected from outskirt of Bhopal, Madhya Pradesh, India. Further, it was identified by the taxonomist. The leaves were shade dried, reduced to coarse powder and stored in airtight container till further use.

2.2 Preparation of extract

The powdered leaves of *Tridax procumbens* about 250 gm were packed in soxhlet apparatus and extracted with ethanol, methanol and distilled water separately, until the completion of the extraction. The extract was filtered while hot, and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely, and later dried in a desiccator.

2.3 Preliminary Phytochemical studies[10-14]

2.3.1 Test for alkaloids

- Dragendorff's test: To 1 ml of the extract, add 1 ml of dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.
- Mayer's test: To 1 ml of the extract, add 1 ml of mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.
- Hager's test: To 1 ml of the extract, add 3ml of Hager's reagent (Saturated aqueous solution of picric acid), yellow coloured precipitate indicates the presence of alkaloids.
- Wagner's test: To 1 ml of the extract, add 2 ml of wagner's reagent (Iodine in Potassium Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

2.3.2 Test for saponins

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins.

2.3.3 Test for Glycosides

- Legal test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.
- Baljet test: To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.
- Keller-Killiani test: 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelein dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.
- Borntrager's test: Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinone glycosides.

2.3.4 Test for carbohydrates and sugars

- Molisch's test: To 2ml of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

- Fehling's test: To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars
- Benedict's test: To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

2.3.5 Test for tannins and phenolic compounds

- Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.
- To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.
- The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

2.3.6 Test for flavonoids

- The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light, formation of fluorescence indicates the presence of flavonoids.
- Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour solution formed, disappears on addition of an acid indicates the presence of flavonoids.
- Shinoda's test: The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.
- The extract is treated with sodium hydroxide, formation of yellow colour indicates the presence of flavones.
- The extract is treated with concentrated H_2SO_4, formation of yellow or orange colour indicates flavones.

2.3.7 Test for steroids

- Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.
- Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H_2SO_4. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

2.3.8 Test for triterpenoids

Noller's test: Dissolve two or three granules or tin metal in 2ml thionyl chloride solution. Then add 1ml of the extract into test tube and warm, the formation of pink colour indicates the presence of triterpenoids.

2.4 In vitro antioxidant activity of extract

2.4.1 Hydrogen-donating activity

The methanol solution of DPPH (100 mM, 2.95 ml), 0.05 ml of extracts dissolved in methanol was added at different concentrations (50-250 µg/ml). Reaction mixture was shaken and after 30 min at room temperature, the absorbance values were measured at 517 nm and converted into percentage of antioxidant activity (%AA). Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract, was calculated by the following equation[15].

$$\% AA = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{DPPH}\}$$

2.4.2 Total polyphenol content

Total polyphenol content was determined using colorimetric method. 1.0 ml of the prepared extract was oxidized using 2.5 ml of Folin- Ciocalteu reagent, and 2.0 ml of sodium carbonate solution (75 g/l) was then added to the reaction mixture. The absorbance readings were taken at 760 nm after incubation at room temperature for 2 h. The amount was calculated using the gallic acid calibration curve. The results were expressed as gallic acid equivalent (GAE) mg per 100 ml of the sample (extract).

2.4.3 Total flavonol content

Flavones and flavonols contents were analyzed by the colorimetric method. 9.8 ml of the prepared extract was mixed with a 10% solution of aluminum chloride (200 µl). After 30 min, absorption was measured at a 425 nm wavelength. The amount was calculated using quercetin calibration curve[16]. The results were expressed as the quercetin equivalent (QE) mg per 100 ml of the sample.

2.4.4 Reducing power assay

The extracts and ascorbic acid were dissolved separately in 1.0 mL of deionized water with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 mL, 10% w/v) were added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared

$FeCl_3$ solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm by making 500 µg mL^{-1} extracts aliquot[17].

2.5 Statistical analysis

Three samples of *Tridax procumbens* plant were independently analyzed and all of the determinations were carried out in triplicate. The results are expressed as means ± standard error means.

3 Result and Discussions

3.1 Phytochemical screening

The qualitative chemical test of ethanol, methanol and aqueous extracts of *Tridax procumbens* leaves exhibited the presence of alkaloids, polyphenol, tannins, flavonoids, carbohydrates, saponin and glycoside (Table 1). The ethanol extract demonstrated the presence of alkaloids, glycosides, carbohydrates, polyphenols, flavonoids and tannins while alkaloids and glycosides were absent in methanol extract. The carbohydrates, polyphenols, flavonoids, saponins and tannins were present in aqueous extracts.

Table 1: Phytochemical analysis of various extracts of *Tridax procumbens* leaves

Test for	Ethanol extract	Methanol extract	Aqueous extract
Alkaloids	+	-	-
Glycosides	+	-	+
Carbohydrates	+	+	+
Polyphenols	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	-	-	+
Triterpenes	-	-	-
Steroids	-	-	-

+ = Present, – = Absent

These classes of phytochemicals are known to possess a variety of biological activities including antimicrobial, antioxidant, anti-inflammatory, antiplasmodial, antidiabeties, hepatoprotective and anticancer activities. The findings of phytochemical screening may partially justify the traditional use of the examined plants in the treatment of various diseases and free radical mediated diseases and indicates that they may serve as a source of bioactive compounds against these illnesses[18].

3.2 Antioxidant activity

3.2.1 Hydrogen-donating activity of extract

DPPH is stable nitrogen centered free radical that can adopt an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals act with appropriate reducing agents, then depriving colour stoichometrically with the number of electrons depleted which is measured spectrophotometricallty at 517 nm[19].

Table 2 demonstrated the DPPH radical scavenging ability of *Tridax procumbens* leaves with different extraction solvents. The differential scavenging activities of the extract against the DPPH system that has been observed could be explained by the presence of different compounds in the extracts. The ethanol extract of *Tridax procumbens* leaves exhibited lowest IC_{50} of DPPH radical scavenging (µg/mL), while the highest IC_{50} belonged to the aqueous extract. Although the DPPH radical scavenging activities of the extracts were less than the ascorbic acid. The ethanol, methanol and aqueous extract of *Tridax procumbens* leaves strongly scavenged DPPH radical with the IC_{50} being 72.06 µg/ml (Fig 1), 96.28 µg/ml (Fig 2) and 117.89 µg/ml (Fig 3), respectively.

Table 2: Free radical scavenging capacity of various extracts of *Tridax procumbens*

Conc. (µg/ml)	DPPH Scavenging %			
	Ethanol Extract	Methanol Extract	Aqueous Extract	Ascorbic Acid
50	38.43±0.82	29.15±0.36	24.47±0.92	96.13±0.49
100	65.17±0.28	52.37±0.47	39.27±0.42	-
150	112.34±0.64	74.62±0.65	64.12±0.67	-
200	173.57±0.41	92.83±0.53	84.17±0.72	-
250	205.73±0.61	121.46±0.28	102.53±0.58	-
IC_{50}	72.06	96.28	117.89	-

Values are mean ± SEM of six determinations

Results showed that DPPH antioxidant activity of the *Tridax procumbens* leaves increased in the following order: ethanol extract > methanol extract > aqueous extract. The ethanol extracts exhibited highest scavenging property compared to other extracts.

3.2.2 Total phenolic content of extract

The ethanol, methanol and aqueous extract of *Tridax procumbens* leaves were evaluated for exploration of the total phenolic content in extracts. Standard curve of gallic acid was calculated and plotted in distilled water for determining absorption data (Fig 4).

From this Beer's law range and regression coefficient is determined. The linear equation of gallic acid was found to be y = 0.0376x - 0.0069 (Fig 4).

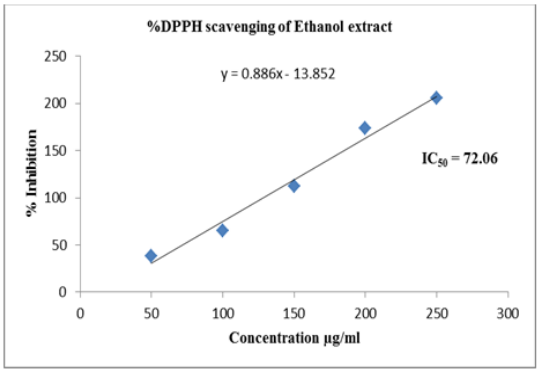

Fig 1: IC_{50} values of ethanol extracts of *Tridax procumbens*

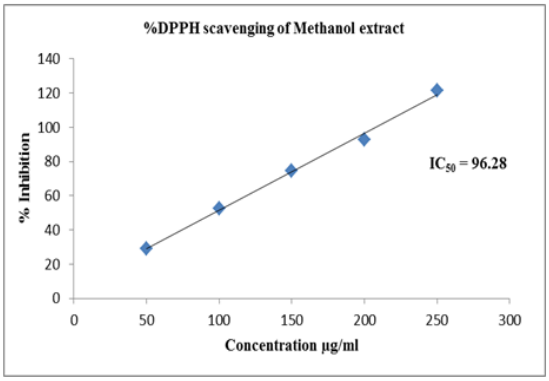

Fig 2: IC_{50} values of methanol extracts of *Tridax procumbens*

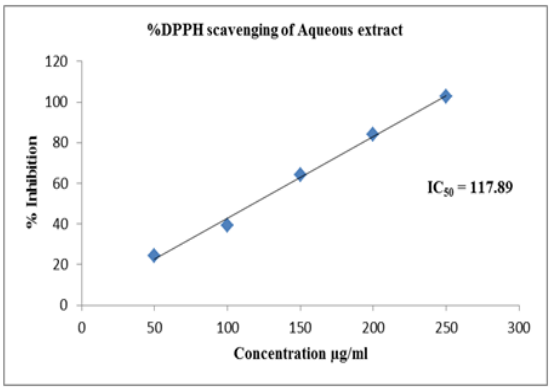

Fig 3: IC_{50} values of aqueous extracts of *Tridax procumbens*

The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method, are depicted in table 3. The total phenolic content in extracts, expressed as gallic acid equivalents. The total phenolic content of ethanol, methanol and aqueous extract of *Tridax procumbens* leaves were 79.53, 65.16 and 59.24 GAE mg/gm, respectively. The ethanol extracts exhibited highest amount of total polyphenol content compared to other extracts.

3.2.3 Total flavonol content of extract

The concentration of flavonoids in ethanol, methanol and aqueous extract of *Tridax procumbens* leaves were determined spectrophotometrically using aluminum chloride. The content of flavonoids was expressed in terms of quercetin equivalents. Standard curve of quercetin was calculated and plotted in distilled water for determining absorption data. From this Beer's law range and regression coefficient is determined. The linear equation of quercetin was found to be y = 0.0375x + 0.0212 (Fig 5).

The content of flavonoids identified in the tested extracts is shown in table 4. The concentrations of flavonoids in ethanol, methanol and aqueous extract of *Tridax procumbens* leaves were 61.28, 55.43 and 42.17 QE mg/gm, respectively. The ethanol extracts exhibited highest amount of flavonoids content compared to other extracts.

Table 3: Determination of total polyphenol content of *Tridax procumbens* extract

Extract	Total polyphenol content (GAE mg/gm)
Ethanol	79.53±0.89
Methanol	65.16±0.47
Aqueous	59.24±0.65

Table 4: Determination of total flavonol content of *Tridax procumbens* extract

Extract	Total flavonol content (QE mg/gm)
Ethanol	61.28±1.02
Methanol	55.43±0.58
Aqueous	42.17±1.12

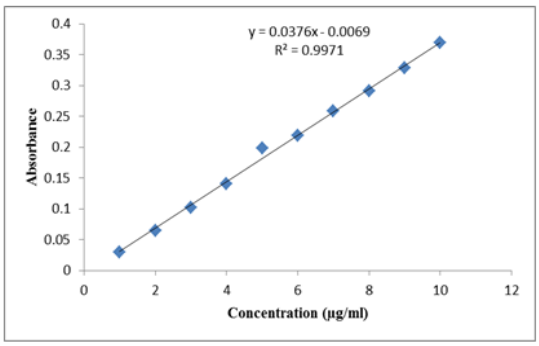

Fig 4: Calibration curve of gallic acid in distilled water

Polyphenol and flavonoids are used for the prevention and cure of various diseases, which are mainly associated with free radicals. It is well known that plant phenolics, in general are highly effective in free radicals scavenging, and they are antioxidants[20]. The findings of total polyphenol and flavonol content of ethanol, methanol and aqueous extract of *Tridax*

procumbens leaves supports the study of DPPH scavenging capacity of extracts.

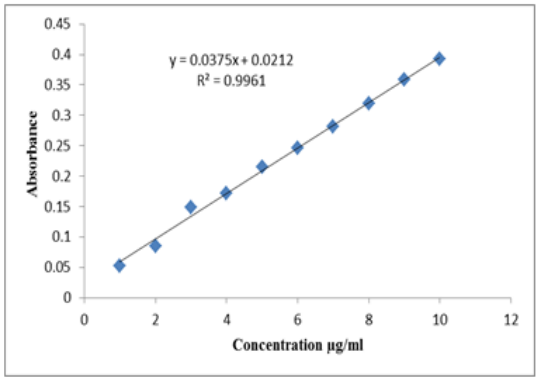

Fig 5: Calibration curve of quercetin in distilled water

As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Flavonoids, including flavones, flavanols and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidants *in vivo*.

The high phenolic and flavonoid content is responsible for the bioactivity of these crude extracts. Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals implicated in several diseases. Flavonoids suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and up-regulate and protect antioxidant defenses. Similarly, phenolics conferring oxidative stress tolerance on plants. Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are increasingly being used in the food industry for their antioxidative properties and health benefits[21].

3.2.4 Reducing power assay of extract

The absorbance value of ascorbic acid was considered to be 100% antioxidant activity. The higher the absorbance of the reaction mixture, the higher would be the reducing power. Table 5 revealed that the antioxidant activity of ethanol, methanol and aqueous extract of *Tridax procumbens* leaves. The reducing power of the ethanol, methanol and aqueous extract of *Tridax procumbens* leaves were found to be 43.81%, 34.70% and 30.21%, respectively.

The reducing power of ascorbic acid was found to be higher than ethanol, methanol and aqueous extracts. It has been reported that the reducing power of substances is probably because of their hydrogen donating ability. The ethanol extract of *Tridax procumbens* might, therefore, contain high amount of reductions than methanol and aqueous extract. The result indicates that extracts act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reactions. The findings indicate that antioxidant activity was produced due to the presence of polyphenol compounds.

Table 5: Antioxidant activity of *Tridax procumbens* extract

Particulars	Absorbance at 700 nm	Antioxidant activity (%)
Ascorbic acid	0.824±0.18	100.00
Ethanol	0.361±0.32	43.81
Methanol	0.286±0.53	34.70
Aqueous	0.249±0.21	30.21

Values are mean ± SEM of triplicate determinations

The reducing power assay is generally used to estimate the ability of an antioxidant to donate an electron which is an important mechanism of phenolic antioxidant action. Earlier many researchers reported that phenolic and flavonol contents of certain plant extracts is directly proportional to the antioxidant activity of extracts. The finding of hydrogen-donating activity as well as total polyphenol and flavonol content implies that antioxidant activities of ethanol extract are maximum compared to other extracts[19].

The results of antioxidant activity indicates that *Tridax procumbens* extracts are a good potential source of secondary metabolite compounds, which can be used as natural antioxidants against free radical oxidative damage in the human body.

4 Conclusion

The presence of the polyphenol and flavonoids in the extract of *Tridax procumbens* leaves makes it pharmacologically active. Their antioxidant activity may be responsible for their usefulness in the management and treatment of various diseases. Further in details phytochemical analysis should be done to identify the active phenolic and flavonoid components present in *Tridax procumbens* leaves.

5 Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

6 Author's contributions

PS and KJ carried out literature review and experimental work of the present study. SK was responsible for statistical work and calculations in addition to manuscript proofing. PS carried out discussion of the present study. All authors read and approved the final manuscript.

7 References

1. Affonso RCL, Voytena APL, Fanan S et al. Phytochemical Composition, Antioxidant Activity, and the Effect of the Aqueous Extract of Coffee (*Coffea arabica* L.) Bean Residual Press Cake on the Skin Wound Healing. Oxidative Medicine and Cellular Longevity. 2016; 2016: 1-10.
2. Strzemski M, Kosior MW, Sowa I et al. *Carlina vulgaris* L. as a Source of Phytochemicals with Antioxidant Activity. Oxidative Medicine and Cellular Longevity. 2017; 2017: 1-10.
3. Tanvir EM, Hossen MS, Hossain MF et al. Antioxidant Properties of Popular Turmeric (*Curcuma longa*) Varieties from Bangladesh. Journal of Food Quality. 2017; 2017: 1-8.
4. Dzialo M, Mierziak J, Korzun U, Preisner M, Szopa J, Kulma A. The Potential of Plant Phenolics in Prevention and Therapy of Skin Disorders. Int J Mol Sci. 2016; 17(2): 160-201.
5. Jain A, Jain A. *Tridax procumbens* (L.): A weed with immense medicinal importance: A Review. International Journal of Pharma and Bio Sciences. 2012; 3(1): 544-552.
6. Vishnu PP, Srinivasa RA. Evaluation of anticancer activity of *Tridax procumbens* leaf extracts on A549 and HEP G2 cell lines. Asian J Pharm Clin Res. 2015; 8(3): 129-132.
7. Ikewuchi CC, Ikewuchi JC, Ifeanacho MO. Phytochemical Composition of *Tridax procumbens* Linn Leaves: Potential as a Functional Food. Food and Nutrition Sciences. 2015; 6: 992-1004.
8. Ikewuchi JC, Ikewuchi CC, Ngozi IM. Chemical profile of *Tridax procumbens* Linn. Pakis J Nutri. 2009; 8(5): 548-50.
9. Saxena VK, Albert S. β-Sitosterol-3-O-β-Dxylopyranoside from the flowers of Tridax procumbens Linn. J Chem Sci 2005:117(3):263-6.
10. Bama SS, Kingsley JS, Sankaranarayanan S, Bama P. Antibacterial activity of different phytochemical extracts from the leaves of *T. procumbens* Linn: Identification and mode of action of the terpenoid compound as antibacterial. Int J Pharm Pharm Sci. 2012; 4(1): 557-564.
11. Harbone JB. Phytochemical Methods: A guide to modern techniques of plant analysis. London: Chapman and Hall Ltd; 1984.
12. Kenwat R, Prasad P, Sahu RK, Roy A, Saraf S. Preliminary phytochemical screening and in vitro antioxidant efficacy of fruit oil of *Martynia annua*. UK Journal of Pharmaceutical and Biosciences. 2014; 2(1): 16-22.
13. Gupta AK, Ahirwar NK, Shinde N, Choudhary M, Rajput YS, Singh A. Phytochemical Screening and Antimicrobial Assessment of Leaves of *Adhatoda vasica, Azadirachta indica* and *Datura stramonium*. UK Journal of Pharmaceutical and Biosciences. 2013; 1(1): 42-47.
14. Singh B, Ahamad A, Pal V. Evaluation of Antibacterial Activity and Phytochemical Screening of *Azadirachta indica* Leaves Extracts Against *Staphylococcus aureus*. UK Journal of Pharmaceutical and Biosciences. 2015; 3(4): 43-47.
15. Sharma U, Sahu RK, Roy A, Golwala DK. *In vivo* antidiabetic and antioxidant potential of *Stephania hernandifolia* in streptozotocin-induced-diabetic rats. Journal of Young Pharmacists. 2010; 2(3): 255-260.
16. Nelofar Gulam Nabi, Mukta Shrivastava. Phytochemical Screening and Antioxidant Activity of Ethanol Extract of *Psoralea corylifolia* seeds. UK Journal of Pharmaceutical and Biosciences. 2017; 5(2): 01-07.
17. Arash KE, Rosna MT, Sadegh M, Behrooz B. Antioxidant Activity and Total Phenolic and Flavonoid Content of Various Solvent Extracts from In Vivo and In Vitro Grown *Trifolium pratense* L. (Red Clover). Bio Med Research International, 2015.
18. Akter K, Barnes EC, Brophy JJ, et al. Phytochemical Profile and Antibacterial and Antioxidant Activities of Medicinal Plants Used by Aboriginal People of New South Wales, Australia. Evidence-Based Complementary and Alternative Medicine. 2016; 2016: 1-14.
19. Sarkar A, Tripathi VD, Sahu RK, Aboulthana WM. Evaluation of Anti-inflammatory and Anti-arthritis Activity of Isolated Fractions from *Bauhinia purpurea* Leaves Extracts in Rats. UK Journal of Pharmaceutical and Biosciences. 2017; 5(1): 47-58.
20. Baba SA, Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. Journal of Taibah University for Science. 2015; 9(4): 449-454.
21. Bravo L. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance Nutr. Rev. 1998; 56: 317-333.