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Estimation of Total Phenolic and Flavonoid content, Antibacterial and Antioxidant Potential of *Tridax procumbens* Linn. from Paschim Medinipur

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ABSTRACT: Since the dawn of time, people have employed plants as a major source of biologically active compounds. Due to toxicity of synthetic drugs and ongoing emergence of bacterial resistance, there is a growing urgency for plant based medication. *Tridax procumbens* L., a small weed of Asteraceae, is widely recognized for its therauptical properties. Investigating phytochemical components (flavonoids and phenolics), antioxidant and antibacterial capability of *Tridax procumbens* L. were the goals of the current study. Using methanol and water as solvents, the phytochemicals were extracted from the plant. Deploying the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) technique, antioxidant activity was assessed. The broth dilution technique was used to find out antibacterial activity. The methanolic extract showed better antioxidant capability compared to the aqueous extract and it also prevented the growth of gram positive bacteria (*Staphylococcus aureus* MTCC 87) at a concentration of 0.8 mg/ml. The findings of this study imply that the plant can be employed as a source of natural antioxidant and antibacterial agent.

Keywords: *Tridax procumbens*, Antioxidant, Antibacterial, Polyphenols, Flavonoids.

INTRODUCTION

Plants have long been used as a source of medication and India's healthcare system heavily relies on them (Prasathkumar *et al*., 2021). Plants have the ability to synthesize chemical compounds that are effective in treating a wide range of human illnesses. These include secondary metabolites which is associated with defense system of plants against microorganisms, insects and herbivores (Bennett and Wallsgrove 1994). Among these, phenols and flavonoids are two classes of secondary metabolites that are capable of removing free radicals and protecting human body against illness caused by oxidative stress (Kumar *et al.*, 2014). Oxidative stress occurs when the synthesis of reactive oxygen species outpaces the antioxidant protection system. In addition to their antioxidant capabilities, these two classes of secondary metabolites are also being investigated for their antibacterial potential due to rapid rise in bacterial resistance against commercially available antibiotics.

Tridax procumbens, a member of family Asteraceae, is an invasive weed with enormous therapeutic potential. The plant is known as "Tridakshya" in many parts of Paschim Medinipur. Leaves of the plant are pinnate and shows opposite phyllotaxy. Capitulam inflorescence is found where ray florets are white and disc florets are yellow in colour. Utilization of *Tridax procumbens* as antimicrobial, antioxidant and mosquito repellent agent is well documented in traditional system of medicine (Kumar *et al.*, 2015; Sabarinath *et al.*, 2020). In Paschim Medinipur, the plant is popularly used as raw material for treating wound infection and its healing (Samanta and Panda 2012). Few works has been done on its pharmacological properties (Andriana *et al.*, 2019; Syed *et al.*, 2020; Reena, 2016) still antioxidant and antibacterial potential based on organic solvent and water is not well addressed. So, this study was conducted to evaluate the antibacterial and antioxidant properties of methanolic and aqueous extract from *Tridax procumbens*.

MATERIAL AND METHODS

Sample Preparation. From several areas of Paschim Medinipur, the plant was collected. The herb was identified and authenticated by Botanical Survey of India, Kolkata. The voucher specimen no. is VU/AB-02. The samples were properly cleaned with tap water to get rid of all the debris. After being air dried for few days, the plant was ground into a fine powder using a grinding machine. The powdered sample was stored for later use in a zip bag that was carefully sealed.

Fig. 1. *Tridax procumbens* L.

Extraction. About 10 grams of dried powder was macerated with 100 ml of methanol and water separately. With the use of Whatman No. 1 filter paper, the liquid extract was filtered. Re-maceration was carried out until the extract turned colourless. After numerous cycles of re-maceration, a semi solid methanolic extract was obtained using rotary evaporator. Aqueous extract was dried with the help of lyophilizer.

Determination of Total Phenolic Content (TPC). With slight modifications, folin-phenol method (Phuyal *et al.,* 2020) was deployed to determine the total phenolic content. The total phenolic content was calculated after putting sprectrophotometrically (760nm) measured absorbance of the sample in the standard curve of galic acid which was prepared with the concentrations of 10, 30, 50, 70, 90 and 100 µg/ml. The total phenolic content was expressed in milligram galic acid equivalent (GAE) per gram of sample (mg/g).

Determination of Total Flavonoid Content (TFC). The aluminium chloride colorimetric method (Phuyal *et al.,* 2020) was slightly modified to measure the total flavonoid content. A volumetric flask was filled with 1 ml sample (1 mg/ml), 4 ml water, 0.3 ml 5% $NaNO_3$, 0.3 ml $AlCl_3$, and 1 ml of 1M NaOH. With distilled water, the final volume in the flask was made upto 10 ml. A standard curve of quercetin was prepared following same procedure. A UV-Vis spectrophotometer was used to measure the absorbance against a blank at 510 nm. In milligrams of quercetin equivalent per gram of sample (mg/g), the total flavonoid content was determined.

In vitro Antioxidant assay. Using a slightly modified version of the DPPH method (Nayak *et al.,* 2020), the extract's antioxidant activity was assessed. For this experiment, extracts at various concentrations (20, 40, 60, 80, and 100 ug/ml) were made from the stock solution (1mg/ml). 0.16Mm DPPH and 1 ml of the extract was mixed in a 2:1 ratio and the mixture was then incubated for 30 minutes. Ascorbic acid was used as the positive control. By measuring the absorbance at 517 nm with a spectrophotometer, the degree of DPPH decolorization was determined. The IC50 value (concentration of test sample required for 50% inhibition) was computed based on the absorbance of each concentration of test sample. The inhibition percentage was calculated using following formula

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

Antibacterial assay. The antibacterial potential was evaluated using broth dilution method (Valgas *et al.,* 2007). The microorganisms selected for this study were *Staphylococcus aureus* MTCC 87, *Pseudomonas aeruginosa* MTCC 741. Single colony of organism, which was picked from a LB streak plate, was dissolved in 3 ml LB broth and incubated overnight at 37 °C, 220 rpm. The bacterial concentration in overnight grown culture was adjusted to 1×10^7 CFU/ml and it was used as an inoculam. All experimental wells containing different concentrations (0.0125, 0.025, 0.05, 0.10, 0.2, 0.4, 0.8 and 1.6 mg/ml) of the test sample was inoculated with 50 µl of log phase culture. An uninoculated broth and broth containing different concentration (same as test sample) of gentamicin was kept as negative control and positive control respectively. The tubes were incubated at 37 °C, for 24h. OD_{600} was checked with a spectrophotometer. The concentration of test sample at which there is no visible bacterial growth (no solution turbidity on naked eyes) was considered as Minimum Inhibitory Concentration (MIC). Absorbance (OD_{600}) of each well was measured keeping the uninoculated broth as blank.

RESULTS

Total phenolic and flavonoid Content. The methanolic extract showed higher phenolic and flavonoid content than the aqueous extract. The result is shown in Table 1 and 2.

Table 1: Total phenolic and flavonoid content of methanolic extract from *Tridax procumbens* L.

Equation obtained from the standard curve	Total phenolic content (mg/gm GAE)	Equation obtained from the standard curve	Total flavonoid content (mg/gm QE)
y = 0.011x - 0.090 R^2 = 0.998	88.72±0.011	y = 0.007x - 0.040 R^2 = 0.991	43.37±0.080

Table 2: Total phenolic and flavonoid content of aqueous extract from *Tridax procumbens* L.

Equation obtained from the standard curve	Total phenolic content (mg/gm GAE)	Equation obtained from the standard curve	Total flavonoid content (mg/gm QE)
y = 0.011x - 0.042 R^2 = 0.998	71.05±0.29	y = 0.007x - 0.040 R^2 = 0.991	29.28±0.430

In vitro Antioxidant assay. Methanolic extract displayed stronger antioxidant potential than the aqueous extract with IC_{50} value of 75.40 µg/ml. The IC_{50} value of aqueous extract and ascorbic acid was 112.19 and 40.48 µg/ml respectively. The result is shown in Table 3.

Antibacterial Assay. Methanolic extract prevented the growth of Staphylococcus aureus MTCC 87 at 0.8mg/ml. The result is displayed in Table 4.

Table 3: Inhibition percentage of different concentrations of methanolic extract, aqueous extract and ascorbic acid.

Concentration (µg/ml)	Aqueous extract	Methanolic extract	Ascorbic acid
20	12.21±0.89	14.05±0.38	29.54±0.49
40	18.14±0.46	28.52±0.15	51.12±0.72
60	25.76±0.55	40.99±0.58	69.64±0.42
80	37.93±0.38	53.32±0.17	85.37±0.58
100	44.98±0.23	64.67±0.32	96.41±0.49

Table 4: Antibacterial activity of methanolic extract, aqueous extract and gentamicin.

Test Organism	Minimum Inhibitory Concentration (MIC)		
	Methanolic extract	Aqueous extract	Gentamicin
Staphylococcus aureus MTCC 87	0.8 mg/ml	>1.6 mg/ml	0.1 mg/ml
Pseudomonas aeruginosa MTCC 741	>1.6 mg/ml	>1.6 mg/ml	0.1 mg/ml

DISCUSSION

The concentration of phenol, flavonoid content and antioxidant potential was comparatively higher in methanolic extract of the plant which indicates that the antioxidant potential is positively correlated with the phenolic and flavonoid content of the extract. According to Prakash *et al.* (2007), phenolic compounds have the ability to scavenge and eliminate free radicals. The results also indicate that using methanol as a solvent can extract more polyphenols from plants. Polyphenols (i.e. phenolic compounds and flavonoids) are thought to be highly effective in preventing a wide range of illnesses caused by oxidative stress like cardiovascular diseases, hepatic disorder, cancer, diabetes and neurological diseases (Suleiman and Ateeg 2020). This result is supported by the work of Do *et al*. (2014) where the methanolic extract exhibited more phenol and flavonoid content than the aqueous extract.

The methanolic extract also showed antibacterial activity which was evaluated using broth dilution technique. At a concentration of 0.8 mg/ml, methanolic extract prevented the growth of *Staphylococcus aureus* MTCC 87. Study of Dhanabalan (2008) came with similar findings where organic solvent extract had stronger antibacterial potential than the aqueous extract.

CONCLUSIONS

According to the current study's findings, methanolic extract of *T. procumbens* exhibit strong antioxidant activity that was found to positively correlate with the phenolic and flavonoid content of the plant. Methanolic extract also showed antibacterial potential against *Staphylococcus aureus*. Based on the results of this study, it can be concluded that *T. procumbens* is a potential source of natural antioxidants. It can also be used to treat skin infections caused by *Staphylococcus aureus* although toxicity and in vivo studies are required to be conducted.

FUTURE SCOPE

Due to antioxidant and antibacterial properties of *T. procumbens*, the plant might be extremely valuable to the food production industries as well as effective in alternative medicine system.

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Conflict of Interest. None.

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