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Research Article

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Anti-oxidant, anti-diabetic, antimicrobial and hemolytic activity of *Tridax procumbens*

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ABSTRACT

Tridax procumbens, is a weed extensively used in traditional medicine as anticoagulant, antifungal and insect repellent. Water extract of leaf and flower contained more proteins when compared to its methanolic counterpart. The same was observed in case of total free phenol present wherein among the methanolic extracts, leaf showed good result when compared to flower. Hydroxyl free radical scavenging assay is an assay to determine the antioxidant property of the sample. Water extract of flower and leaf gave the maximum hydroxyl scavenging activity of 33.5±1.2% and 27.32±0.98%. Methanolic extract of flower followed this result 22.5±2.14% where methanolic leaf extract gave 17.25±1.34%. Methanolic extract of flower gave a maximum α-amylase inhibition of 20.7±2.54% at pH 3 wherein the water extract of flower gave maximum at pH 7. This was followed by water extract of leaf (pH 3) and its methanolic extract (pH 3). P.aragenosa was found to be more susceptible to the extracts when compared to other microorganisms. Methanolic extract of flower was found to inhibit P.aragenosa, E.coli, and B.cereus. wherein methanolic extract of leaf gave maximum zone against P.fluroscence. Methanolic extracts alone were found to have haemolytic activity wherein the methanolic flower extract gave a zone of 3±0.05 mm and methanolic leaf extract gave 2±0.01 mm. Water extract of the samples did not give any zones and were found to be safe for haemocytes. Based on the studies, Tridax procumbens can be incorporated in medicines and nutraceuticals for increasing their effectiveness.

Keywords: antioxidant, antidiabetic, haemolytic, antimicrobial, *Tridax*

INTRODUCTION

Tridax procumbens, known as *Tha-Thachedi* or *Vettukaayapoondu* in tamil is a weed extensively used in traditional medicine as anticoagulant, antifungal and insect repellent. *Trida*x is a weak herb of about10-30cm in length with leaves about 4-6cm long andhas two types of flowers namely ray-florets and disk-florets while its fruit is cypsela [1].

The leaf and flower of *Tridax procumbens* possesses antiseptic, insecticidal and antiparasitic properties. It is also known to prevent hair fall and check hemorrhage from cuts and bruises. They also possess various pharmacological activities like immunomodulatory, antidiabetic, anti-hepatotoxic and anti-oxidant, Anti-inflammatory and Analgesic activity [2]. The most important of the bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [3]. The extracts of *Tridax procumbens* have been reported to have antibacterial properties [4]. Whole plant of *Tridax* has reported for its antimicrobial activity on various species of bacteria. A whole plant is squeezed between the palms of hands to obtain juice [5].

EXPERIMENTAL SECTION

Collection of Plants

Plants were collected from virgin land of P.S.R. Engineering College, washed to remove dust and dirt. leaves and flowers were shade dried, powdered using mortar and pestle.

Extraction

30g of samples were dissolved in 50ml of water and methanol respectively and left on shaker for 48hours. the extracts were filtered and lyophilised to get extract in powder form. 1mg/ml of the extract in water was used as stock for further studies.

Estimation of total crude protein

Total crude protein in the extract was determined by Lowry's Method. 0.5 ml of extract was made to 1ml using distilled water and 5ml of reagent was added. This was incubated at room temperature for 10 minutes. 0.5 ml of Folin's reagent was added to the mixture and incubated at room temperature for 20 minutes. absorbance was taken at 660nm and compared with BSA Standard.

Estimation of total free phenol

Total free phenol in the extract determines the overall antioxidant activity. This assay was performed by modifying Folin-Ciocalteau method [6]. 0.5 ml of extract was mixed with 4ml of 1molar Na_2CO_3 and 0.5ml of 0.1 molar Folin's reagent. The mixture was incubated at 70°C for 30 minutes and absorbance was determined by colorimetric method at 765nm. Galic acid was used as standard

Hydroxyl free radical scavenging activity

Hydroxyl freeradical scavenging ability was determined by a method described by Farhadi, K., et al, [7] with slight modification. 1.5ml of extract was mixed with 20 µl 30% H_2O_2 solution. absorbance was read at 530 nm at time intervals of 5 minutes for 1 hour. decrease in absorbance indicates increasing scavenging ability.

α-Amylase inhibition activity

α-amylase inhibition by the extract was determined using Bernfield method [8]. 0.5 ml of extract and 0.5 mg/ml of α-amylase solution (varying pH) were incubated at 25°C for 30 minutes.0.5 ml of Starch solution was added to the reaction mixture and incubated at 25°C for 20 minutes and reaction was stopped using 1ml of DNSA. The mixture was then incubated at 90°C for 5 minutes and cooled to room temperature. The reaction mixture was diluted by addition of 10ml distilled water. Absorbance was measured at 540 nm. α-amylase inhibition activity was calculated as Percentage inhibition.

Antibacterial activity

Antibacterial activity of leaf and flower methanolic and water extracts were analysed by agar well diffusion method. Overnight culture of test bacterial strains were inoculated onto Nutrient agar medium, wells were punched using sterile cork borer and 100µl of extracts were poured into the well and incubated for 24 hours at 37°C. Zones of inhibition was measured and recorded.

Haemolytic activity

0.5ml of sheep RBC collected from local slaughter house was added to 50ml 1% agar (pH 7.5). 0.5 ml of yellow egg yolk was added and poured into sterile Petri plates. wells were bored using sterile cork borer. 100 µl of extracts were added and the plates were incubated at 37°C for 24 hours. Appearance of zones implies haemolytic activity[9].

RESULTS AND DISCUSSION

1mg/ml stock solution was studied for various properties such as total protein content, total free phenol, free radical scavenging, α-amylase inhibition, antimicrobial and haemolytic activity.

Table 1: Total protein and total free Phenols in samples

	TOTAL FREEPROTEINS (g BSAE)	TOTAL FREE PHENOLS (g GAE)
Leaf Methanol	0.83± 0.01	0.132± 0.11
Leaf water	2.92± 0.03	0.282± 0.15
Flower water	2.26± 0.09	0.172± 0.08
Flower methanol	0.94± 0.12	0.082± 0.04

Water extract of leaf and flower contained more proteins when compared to its methanolic counterpart. The same was observed in case of total free phenol present wherein among the methanolic extracts, leaf showed good result when compared to flower.

Hydroxyl free radical scavenging

Hydroxyl free radical scavenging assay is an assay to determine the antioxidant property of the sample. Water extract of flower and leaf gave the maximum hydroxyl scavenging activity of 33.5±1.2% and 27.32±0.98%. Methanolic extract of flower followed this result 22.5±2.14% where methanolic leaf extract gave 17.25±1.34%. Based on the results water extracts have superior hydroxyl scavenging activity when compared to methanolic extracts.

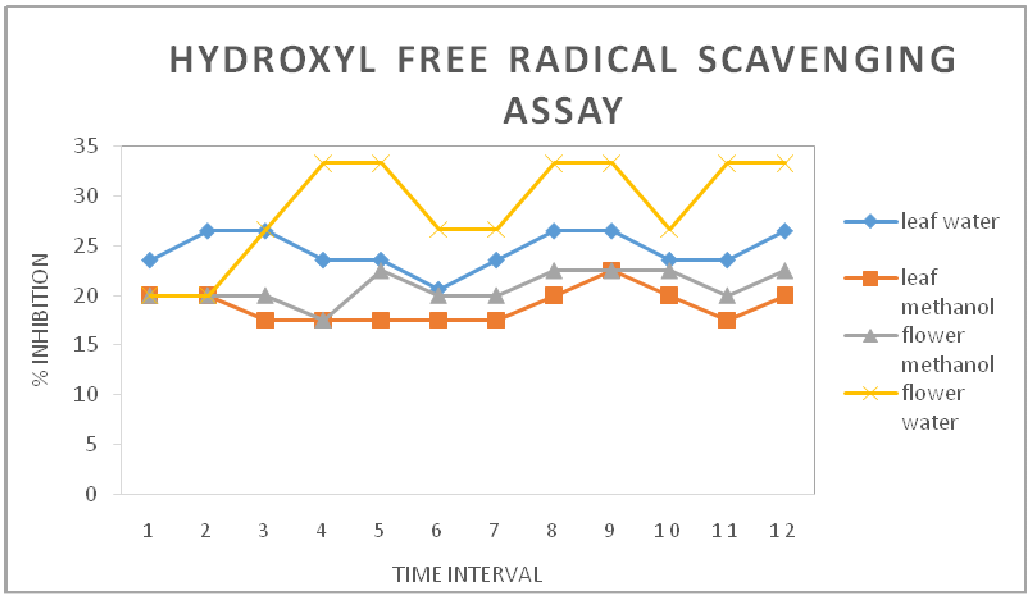

Figure 2: Hydroxyl free radical scavenging assay

α-Amylase Activity

α-amylase is a vital enzyme for Type-II diabetes. Inhibition of pancreatic α-amylase is useful in reducing the effect of Type-II Diabetes. Methanolic extract of flower gave a maximum α-amylase inhibition of 20.7±2.54% at pH 3 wherein the water extract of flower gave maximum at pH 7. This was followed by water extract of leaf (pH 3) and its methanolic extract (pH 3).

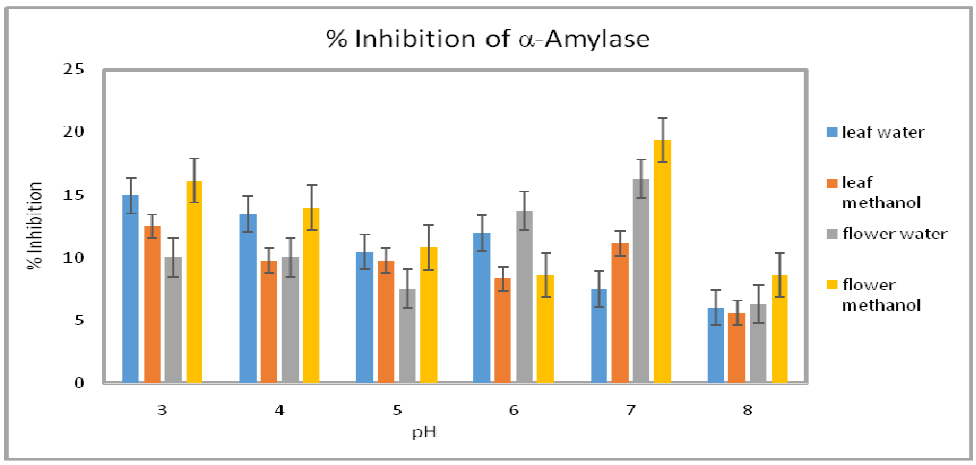

Figure 2: % Inhibition of α-Amylase

Antimicrobial Activity

Table 2: Antimicrobial activity for different organisms

Microorganism	Radius of Zone			
	Leaf Methanol	Leaf Water	Flower Methanol	Flower Water
E. Coli	0.9 ± 0.01	0.6 ± 0.01	1.3 ± 0.02	0.7± 0.01
B.cereus	0.8 ± 0.032	0.6 ± 0.01	0.9 ± 0.02	0.8 ± 0.04
P.fluroscence	1.1 ± 0.008	0.3 ± 0.006	1 ± 0.01	0.8 ± 0.02
P.aragenosa	1 ± 0.01	0.8 ± 0.01	1.9 ± 0.028	1.2 ± 0.03

4 different microbial samples were used to study the antimicrobial activity *T.porcumbens* extract. *P.aragenosa* was found to be more susceptible to the extracts when compared to other microorganisms. Methanolic extract of flower was found to inhibit *P.aragenosa, E.coli,* and *B.cereus.* wherein methanolic extract of leaf gave maximum zone against *P.fluroscence.*

Haemolytic activity

Haemolytic activity of a sample implies its toxic effect on haemocytes. Methanolic extracts alone were found to have haemolytic activity wherein the methanolic flower extract gave a zone of 3±0.05 mm and methanolic leaf extract gave 2±0.01 mm. Water extract of the samples did not give any zones and were found to be safe for haemocytes.

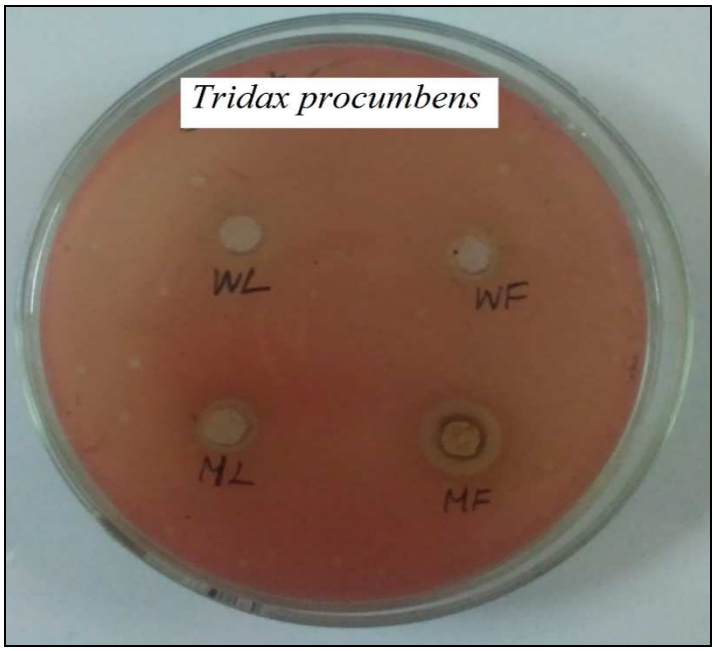

Figure 3: Haemolytic activity

CONCLUSION

Based on the present study, methanolic flower extract of *Tridax procumbens* was found to have good activity against microbes studied and effective antidiabetic activity when compared to the methanolic leaf extract. Water extracts had an activity against microbes, hydroxyl free radical and α-amylase. Water were also found to be safe when compared to their methanolic counterpart.

Based on the studies, *Tridax procumbens* can be incorporated in medicines and nutraceuticals for increasing their effectiveness.

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