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Phytochemical and Biological Investigation of the Leaves of *Tridax procumbens*

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Authors' contributions

All authors were assisted equally to the study and approved the final version of the manuscript.

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ABSTRACT

Aims: Because of various traditional uses of *Tridax procumbens* leaves, the authors choose the plant for investigating the numerous biological activities. The main objective for this study is to search antioxidant activity, hypoglycemic activity and antibacterial activities with initial phytochemical investigations of the leaves of *Tridax procumbens.*
Methodology: The phytochemical purposes were achieved by using two different chromatographic techniques thin layer chromatography (TLC), vacuum liquid chromatography (VLC). DPPH test, glucose uptake activity of yeast cells and agar plate diffusion technique were performed to evaluate the antioxidant capacity, hypoglycemic activity and anti-bacterial properties respectively of different fraction obtained from VLC by using different solvent systems such as dichloromethane, butanol, ethyl acetate and methanol. 31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml and 500 µg/ml sample were used from each fraction to investigate antioxidant and hypoglycemic activity where vitamin C tablet and metformin were used as standard respectively. Antibacterial capacity was measured by

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measuring zone of inhibition resulted by the fractions where ciprofloxacin was taken as reference drug.

Results: Some visible spots were identified in the TLC plate which confirms the presence of different compounds in the fractions obtained from the methanolic extract of the leaves of *Tridax procumbens*. Butanol and ethyl acetate fractions showed the highest antidiabetic activity. In addition to, ethyl acetate, butanol and methanol fractions exerted more than 80% antioxidant capacity in DPPH assay. Furthermore, butanol fraction gives maximum zone of inhibition up to 21 mm against *Staphylocuccos aureas* which ensure anti-bacterial properties of the leaves of *Tridax procumbens* especially against *Staphylocuccos aureas* and its strains.

Conclusion: Notwithstanding the all the research, the leaves had anti-inflammatory properties, which may have been generated by their antioxidant activity. As a result, if more therapeutic research is done, the separated compounds in those fractions may be employed as future therapeutic strategies.

Keywords: Tridax procumbens; hypoglycemic; antidiabetic; anti-oxidant; antibacterial; phytochemistry.

1. INTRODUCTION

Medicinal plants have been recognized and utilized throughout human history owing to their ability to produce a diverse number of chemical compounds that serve significant biological activities and have been used in health and medicinal purposes for thousands of years [1-3]. *Tridax procumbens* Linn. is commonly known as "Coatbutton" or “Tridhara Flower” or "Tridax Daisy" which is a member of the Asteraceae family [4-6]. It grows to a height of 30 to 50 cm and has hairy, short, blade-like leaves. Its thinly hairy, branched stem roots at the nodes [7,8]. Traditionally its leaf is used as a drink to cure treat bronchial catarrh, diarrhea, dysentery, and liver diseases in many countries in Africa, South and Southeast Asia [6, 9-13]. Table 1 shows the taxonomic classification of *Tridax procumbens* [14,15]. Earlier researchers reported presence of a number chemical compounds such as alkaloids, flavonoids, carotenoids, β-sitosterol, fumeric acid, luteolin, quercetin, oxoester, lauric acid, myristic, dexamethasone, glucoluteolin, palmitic, arachidic, linoleic acid and tannin etc. were separated & purified from the plant [16-18]. Linolenic acid and a new flavonoid like compound Procumbenetin (3, 6- dimethoxy-5, 7, 2’, 3’, 4’- pentahydroxyflavone 7- O-β-glucopyranoside) has been reported and isolated from aerial parts of plant, also, a pair of water soluble polysaccharides was purified from the leaves [17,19]. The leaves also contain high amount of minerals for example, calcium, magnesium, potassium, sodium, selenium, etc. and it serves as a good source of plant protein and potassium supplement, carotenoids to the local generation [19-21]. Furthermore, Many bioactive compounds, such as procumbetin [6], 8,3′-dihydroxy-3,7,4′-trimethoxy-6-O-β-d-glucopyranosyl flavone, 6,8,3′-trihydroxy-3,7,4′-trimethoxyflavone; puerarin [22], centaurein, centaureidin, phenolic acids [23] and various lipid constituents [16] such as methyl 14-oxooctadecanoate, methyl 14-oxononacosanoate, 30-methyl-28-oxodotriacont-29-en-l-oic acid, β-amyrone, β-amyrin; lupeol, and fucosterol have been successfully isolated from this plant [15, 24-28].

The aim of this research project was to carry out the characterization of the functional molecules present in the methanolic extract of leaves of the *Tridax procumbens* and investigate their biological activities.

Table 1. Taxonomic Classification of *Tridax procumbens* Linn [14,15]

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Division	:	Spermatophyta
Subdivision	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Asteridae
Order	:	Asterales
Family	:	Asteraceae
Genus	:	*Tridax*
Species	:	*procumbens*

2. MATERIALS AND METHODS

2.1 Plant Materials Collection, Processing, and Phytochemical Investigations

The plants were collected by the students of Pharmacy department, East West University

from Jhalokathi district, under Barishal division of Bangladesh by uprooting the whole plant, then the leaves were removed carefully and voucher specimens have been deposited in Bangladesh National Herbarium (BNH) for future references (DACB Accession Number: 65297). After one week of shade drying, the leaves were grinded to coarse powder and successively extracted with methanol by maceration technique. After subsequent evaporation of solvents 22.5 g methanolic extract of *Tridax procumbens* leaves was obtained. Vacuum liquid chromatography (VLC) was applied collect different fractions in Dichloromethane ($CHCl_2$), Butanol (C_4H_9OH), Ethyl Acetate ($C_4H_8O_2$) and Methanol (CH_3OH) from the crude methanolic extract and marked the fractions as DCMF, BF, EAF & MF respectively. After concentrating the fractions by using rotary evaporator, they were treated with thin layer liquid chromatography. The most active fractions having the similar thin layer chromatography profile were pooled together which were conducted by three solvent systems such as non-polar (Benzene : Ethanol : Aluminum hydroxide = 9 : 1 : 0.1), intermediate polar (Chloroform : Ethyl acetate : Formic acid = 5 : 4 : 1), and polar (Ethyl acetate : Ethanol : Water = 8 : 1.2 : 0.8). After numerous trial and error, non-polar solvent was fixed as mobile phase of thin layer liquid chromatography.

2.2 Anti-oxidant Activity Test

The antioxidant properties of the fractions investigated by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) which was previously describe as the most popular, quickest and easiest approach for the measurement of antioxidant properties [29]. At first, 3.125 mg/ml sample solution was prepared from each fraction with methanol, and 1ml, 2ml, 4ml, 8ml and 16ml of sample solution from each fraction were transferred into five different volumetric flasks and diluted up to 10 ml with methanol. 3 ml of a 0.0004% DPPH solution was added to 1 ml of the solution, which was then diluted with methanol to make it up to 10 ml. After keeping them for 30 minutes in a dark room, the absorbance was measured at 517 nm in a UV spectrophotometer. The standard was a vitamin C tablet (250 mg), and the blanks were methanol and a DPPH mixture. Antioxidant activity was measured using the following equation:

$$Antioxidant\ capacity\ (\%) = \left(1 - \frac{Abs.of\ sample - Abs.of\ blank}{Abs.of\ control}\right) x\ 100$$

2.3 *In vitro* Hypoglycemic Activity Test

Antidiabetic activity was investigated by the previously described method of glucose uptake in yeast cells [30-32]. A 50% (v/v) suspension of commercial baker's yeast was made in distilled water after being repeatedly centrifuged (3,000rpm; 5 min) until the supernatant fluids were clear. 1 ml of glucose solution (10 mM) and various quantities of isolated components (1 mg) were combined, and the mixture was then incubated at 37 °C for 10 minutes. The reaction was initiated by adding 100µl of yeast suspension and incubated for further 60 min at 37 °C and then they were centrifuged at 2,500rpm for 5 min to determine the amount of glucose in the supernatant. Metformin was used as standard and a mixture of all of the chemicals, excluding the test sample were used as control. Glucose uptake was measured by using the following formula:

$$Glucose\ Upatake\ (\%) = \left(\frac{Abs.of\ sample - Abs.of\ control}{Abs.of\ sample}\right) x\ 100$$

2.4 Antimicrobial Screening Test

The agar diffusion assay is one of the established method for quantifying the ability of antibiotics to inhibit bacterial growth [33,34]. The tested bacterium is inoculated on an agar plate first, and then an antimicrobial agent is transferred by diffusion resulting growth inhibition zones [34]. About 100µl of each fraction was added to previously incubated bacterial inoculum containing agar plates. After 30 min refrigeration, and incubation at 37°C for 18 hours, the antimicrobial activity was measured by measuring the zone of inhibition appeared after the incubation period. 10% Methanol was used as a negative control and ciprofloxacin (30µg/disk) was used as the reference standard.

3. RESULTS

3.1 Phytochemical Investigations

After charring of the TLC plate with 10% sulfuric acid solution and DPPH solution some visible spots were observed.

3.2 Anti-oxidant Activity Test

The illustrations of antioxidant test of different fraction of methanolic extract of the *Tridax procumbens* leaves are given Table 3.

Table 2. R_f value of different fractions

Name of sample	1st spot R_f value	2nd spot R_f value	3rd spot R_f value
Crude Extract	0.31	0.50	0.75
DCMF	0.53	0.77	0.89
BF	0.55	0.75	0.96
EAF	0.60	0.87	0.93
MF	0.2	0.5	No spot

Table 3. Anti-oxidant activity test results

Concentration (µg/ml)	Antioxidant Capacity (%)				
	DCMF	BF	EAF	MF	Metformin
31.25	12.61	19.80	62.23	57.56	58.89
62.5	13.28	42.52	72.43	70.25	60.73
125	89.72	47.86	80.78	79.28	63.40
250	40.68	51.89	83.62	81.95	66.75
500	70.25	55.89	84.62	83.29	71.90

Table 4. Antibacterial activity test results

Tested bacteria	Zone of inhibition (mm)					
	MF	EAF	DCF	BF	STD	Control
S. paratyphi	9	9	8	14	24	0
Bacillus sereus	8	9	7	7	23	0
Bacillus subtilis	13	11	8	9	26	0
Staphylocuccos aureas	13	12	9	21	30	0
Salmonela typhi	9	8	8	11	31	0
Shigella dysentriae	10	8	10	7	27	0
Vibrio mimicus	9	9	8	14	28	0
Candida albicans	9	10	9	11	27	0
Aspergillus niger	9	10	9	11	27	0
E.coli	10	11	9	11	25	0
Vibrio parahemoliticus	11	9	8	13	30	0
Bacillus megaterium	13	11	8	9	27	0
Pseudomonas aureaus	11	12	9	12	22	0

Table 5. Hypoglycemic activity test results

Concentration (µg/ml)	Glucose uptake capacity (%)				
	DCMF	BF	EAF	MF	Metformin
31.25	7.68	12.07	13.83	12.07	22.37
62.5	15.25	27.39	20.46	21.9	28.19
125	26.56	52.13	39.01	22.89	33.2
250	33.72	60.41	45.12	29.95	35.74
500	46.03	62.75	58.44	51.22	39.74

3.3 Antibacterial Activity Test

The result of antibiotic test of different fraction of methanolic extract of the *Tridax procumbens* leaves are given Table 4.

3.4 Hypoglycemic Test

The result of hypoglycemic test of different fraction of methanolic extract of the *Tridax procumbens* leaves are given Table 5.

4. DISCUSSION

TLC plates were developed with dichloromethane (DCF), butanol (BF), ethyl acetate (EAF), methanol (MF) fractions by using non polar to most polar solvent system and the best result was found in non-polar solvent system. After charring of the sulfuric acid and DPPH solution on the TLC plate, the visible spots indicate the presence of different compounds in the

methanolic extract of the leaves of *Tridax procumbens.* Further extractions and purifications from these crude drugs may lead to the possible isolation of these compounds from the crude extracts.

In the Glucose uptake in Yeast cells method the mechanism of glucose transport across the yeast cell membrane has been receiving attention as in vitro screening method for hypoglycemic effect of various compounds. It is reported that, in yeast cells (*Saccharomyces cerevisiae*) glucose is transported by a facilitated diffusion process [35]. In our result, It has shown that, butanol fraction (BF) and ethyl acetate fraction (EAF) of methanolic extract give higher antidiabetic activity 62% and 58% respectively. Whereas, methanol fraction (MF) and dichloromethane fraction (DCF) of methanolic extract of give 51% and 46% respectively.

The reduction in DPPH radical which can accept an electron of hydrogen radical was determined by the decrease of its absorbance at 517 nm (in methanol) confirmed antioxidant capacity of the samples [36]. In our result, it has shown that the fractions of dichloromethane (DCF), butanol (BF), ethyl acetate (EAF) and methanol (MF) give respectively 89%, 55%, 84%, and 83% antioxidant activity.

Various strains of gram positive and gram negative bacteria were used as the test microorganism to determine antibacterial capacity of the fractions where the zones of inhibition for the microbes were measured in millimeter by using a transparent ruler. The fraction obtained through butanol (BF) gives zone of inhibition up to 21mm against *Staphylocuccos aureas* which is maximum than others and it indicates that, the leaves of *Tridax procumbens* possessing antimicrobial activity can be employed against human pathogens specially for *Staphylocuccos aureas* and its strains.

5. CONCLUSION

The current study on the different fraction of methanolic extract of the *Tridax procumbens* leaves showed the potentiality as an antioxidant, a hypoglycemic agent and antibacterial agent. Additionally, the leaves displayed anti-inflammatory properties, which may have been triggered by their antioxidant properties. If further therapeutic research is conducted, the isolated compounds in those fractions may be used as future therapeutic tools.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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