Antibacterial and Antifungal potential of *Tridax procumbens*

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Abstract:

Plants are known as producers and hence are also considered as huge reservoirs of natural compounds. These natural compounds play very dynamic roles in various industries like food, cosmetology, pharmaceuticals etc. Several plants belonging to the Asteraceae family like Ageratum sp. Vernonia sp. etc. are known for their medicinal importance. *Tridax procumbens* (commonly known as coat button) also belongs to Asteraceae family. It is a common weed. Since it belongs to the Asteraceae family it is expected to exhibit anti-inflammatory, antioxidant, anti-bacterial and anti-fungal activity, which can be of pharmaceutical importance. The Asteraceae members are well known for producing a variety of secondary metabolites like polyphenols, terpenoids, alkaloids, tannins, flavonoids etc. Various alcoholic and aqueous extracts of stem and leaves of the plants were prepared and the phytochemical analysis of the same was performed and compared. The qualitative analysis for secondary metabolites was performed using invitro testing methods. The antioxidant properties of the extracts were determined using DPPH assay. The antibacterial and antifungal properties of the plant extracts were also determined.

Keywords: Asteraceae, *Tridax procumbens*, Antioxidant properties, Antibacterial properties, Antifungal properties, Phytochemical analysis.

INTRODUCTION

Use of medicinal plants in the treatment and prevention against many infections and diseases are gaining attention worldwide due to its safety. Medicinal plants pose low risk of side effects as compared to the chemical compounds used in treatment [2]. Asteraceae family is widely known for their various secondary metabolite productions like polyphenols, terpenoids, alkaloids etc. which is also of pharmaceutical importance [6]. The metabolites produced by plants of this family are known to have anti-bacterial, anti-fungal, insecticidal, anti-tumor, antioxidants etc [6] [7]. The plant Tridax procumbens also belongs to Asteraceae family. Hence, it is expected to have pharmaceutical importance.

Botanical Classification of *Tridax procumbens:*

Kingdom: Plantae
Division: Spermatophyta
Sub Division: Angiospermae
Class: Dicotyledonae
Sub Class: Gamepetalae
Cohort: Asterales
Family: Asteraceae
Genus: *Tridax*
Species: *procumbens*

MATERIALS AND METHODS

Collection and Authentification of plant sample:

The plant *Tridax procumbens* was collected from the local areas around Mumbai city. Further the authentification of the collected plant sample was performed by herbarium Blatter at St. Xaviers College, Mumbai.

Preparation of plant extracts:

The leaves and stem of the plant *Tridax procumbens* was collected and disinfected. This plant material was further dried and powdered. Homogenization of 10gm of the dried powder was performed using 100ml of solvent (methanol, ethanol and distilled water). This mixture is then placed onto rotatory shaker for overnight at 40 rpm. The crude extract obtained is filtered using Whatman filter paper no.1. The filtered extract was then subjected for concentration using rotatory evaporator at $40^{O}C$ if needed. [5] [7].

Invitro analysis of phytochemicals:

Flavonoids test: Take 1gm of powdered extract in test tube and add few drops of dil. NaOH. An intense yellow coloration will be observed. Add few drops of dil. HCL to the tube. The colour change to colourless will indicate the presence of flavonoids [4] [5].

Alkaloids test: Take 0.1gm of powder extract in test tube and dissolve it in few drops of 2N H_2SO_4. Addition of 2-3 drops Dragendorff reagent, and the appearance of orange red sediment, indicates the presence of alkaloids in the tested sample [5].

Saponins test: Take 0.1gm of extract in test tube and add 10ml of distilled water to it. Shake the mixture for 10-30 sec and check for the appearance of foam on the top of the solution. If the foam is retained for 30 sec or more then it indicates the presence of saponins in the tested sample.
[4] [5].

Steroids test: Take 1mg of extract in test tube and add 10ml of chloroform to it and mix until it dissolve. Add 10ml of conc. H_2SO_4 from the sides of the test tube. Observe for the appearance of red coloration in the upper layer and yellow coloration with green fluorescence in sulphuric acid layer which indicates the presence of steroids [4].

Terpenoids test: The extract was mixed with 2ml of chloroform and concentrate H_2SO_4 (3ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of presence of terpenoids. [1].

Determination of antioxidant activity:

0.1mM of DPPH reagent was used to determine the presence of antioxidants in the prepared alcoholic and aqueous extract. 0.1ml of sample was added to 2.9ml of DPPH reagent and the mixture was incubated in dark for 30min at room temperature. The absorbance of mixture was

recorded at 518nm using UV-Visible spectrophotometer [2]. The radical scavenging activities of the extracts were calculated using following formula:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Absorbance of blank- Absorbance of test}}{\text{Absorbance of blank}}$$

Antibacterial and antifungal activity:

The antibacterial and antifungal activities of extracts were tested using Nutrient broth and Sabourauds broth medium respectively. The extract was tested against bacterias like *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The extracts were tested against fungus like *Aspergillus niger* and *Penicillium chrysogenum.* The O.D of the bacterial and fungal cultures was adjusted to 0.3. 0.1ml of respective cultures of were added to the 5ml of medium along with 0.3ml of respective extract.

RESULTS AND DISCUSSIONS:

Phytochemical Analysis: The leaf and stem extracts of alcoholic and aqueous solvent showed the presence of various classes of secondary metabolites like Flavonoids, Alkaloids, Terpenoids etc

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Phytochemical analysis of leaf extract	Ethanolic Extract	Methanolic Extract	Aqueous Extract
Flavonoids	+	+	+
Terpenoids	+	+	+
Alkaloids	-	+	-
Steroids	+	-	+
Saponins	-	-	-

Table 1: Phytochemical analysis of leaf extracts

Based on the results obtained in table 1, the ethanolic and aqueous extract extracts of leaves showed the presence of flavonoids, terpenoids and steroids, whereas methanolic extract of the leaves showed the presence of flavonoids, terpenoids and alkaloids.

Phytochemical analysis of stem extract	Ethanolic Extract	Methanolic Extract	Aqueous Extract
Flavonoids	+	+	+
Terpenoids	+	+	-
Alkaloids	-	-	-
Steroids	+	+	-
Saponins	-	-	-

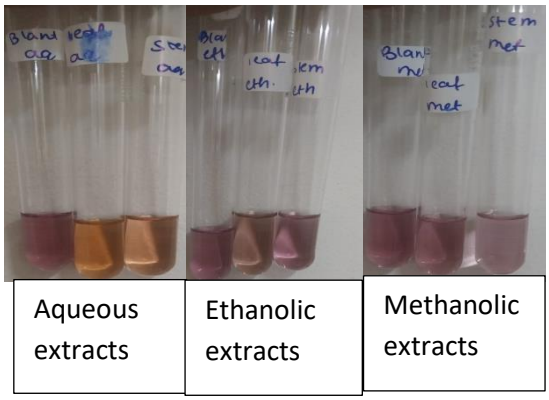
Table 2: Phytochemical analysis of stem extracts

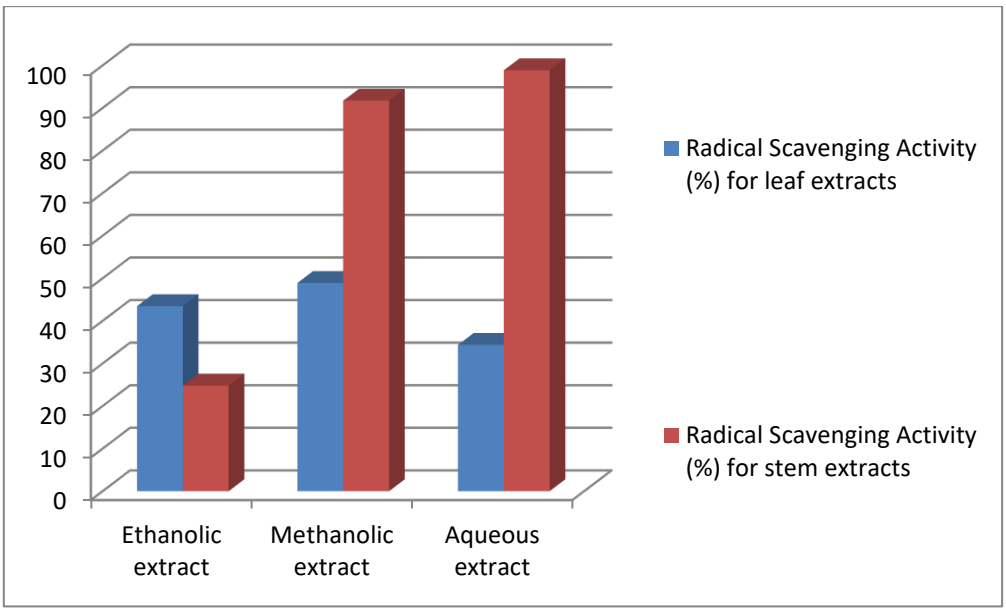
Based on the results obtained in table 2, the ethanolic and methanolic extract extracts of stem showed the presence of flavonoids, terpenoids and steroids, whereas aqueous extract of the stem showed the presence of only flavonoids.

Antioxidant activity: The alcoholic and aqueous extracts of leaves and stem exhibits antioxidant activity that are illustrated as radical scavenging activity

Plant Extracts	Radical Scavenging Activity (%) for leaf extracts	Radical Scavenging Activity (%) for stem extracts
Ethanolic	**43.45**	**24.80**
Methanolic	**48.90**	**91.70**
Aqueous	**34.35**	**98.85**

Table 3: Radical scavenging activity of leaf and stem extracts





Based on the results obtained in table 3, methanolic and aqueous extract of stem showed high percentage of radical scavenging activity, whereas ethanolic extract of leaf showed high percentage of radical scavenging activity as compared to stem.

Antibacterial Activity of stem and leaves extract

Extracts	Cultures	Results (leaf)	Results (stem)
Aqueous	***E.coli***	+	+
	P. aeruginosa	+	+
	S. aureus	+	+
	K. pneumoniae	+	+
Ethanolic	***E.coli***	-	-
	P. aeruginosa	+	+
	S. aureus	+	+
	K. pneumoniae	+	+
Methanolic	***E.coli***	+	+
	P. aeruginosa	+	+
	S. aureus	+	+
	K. pneumoniae	+	+

Table 4: Antibacterial activity of alcoholic and aqueous leaves and stem

Extracts (Leaf and stem)	Controls			
Ethanolic	**PC (37^{O}C)**	**NC (4^{O}C)**	**MC (37^{O}C)**	**SOL (37^{O}C)**
Results	+	-	-	-
	SOL-E.c (37^{O}C)	**SOL-K.p (37^{O}C)**	**SOL-P.a (37^{O}C)**	**SOL-S.a (37^{O}C)**
Results	+	+	+	+
Methanolic	**PC (37^{O}C)**	**NC (4^{O}C)**	**MC (37^{O}C)**	**SOL (37^{O}C)**
Results	+	-	-	-
	SOL-E.c (37^{O}C)	**SOL-K.p (37^{O}C)**	**SOL-P.a (37^{O}C)**	**SOL-S.a (37^{O}C)**
Results	+	+	+	+
Aqueous	**PC (37^{O}C)**	**NC (4^{O}C)**	**MC (37^{O}C)**	**SOL (37^{O}C)**
Results	+	-	-	-
	SOL-E.c (37^{O}C)	**SOL-K.p (37^{O}C)**	**SOL-P.a (37^{O}C)**	**SOL-S.a (37^{O}C)**
Results	+	+	+	+

Table 5: Activity of controls tubes maintained at various condition for antibacterial activity.

E.c	***Escherichia coli***
P.a	***Pseudomonas aeruginosa***
S.a	***Staphylococcus aureus***
K.p	***Klebsiella pneumonia***
SOL	**Solvent Control**
PC	**Positive Control**
NC	**Negative Control**
MC	**Media Control**
RT	**Room Temperature**
+	**Growth**
-	**No Growth**

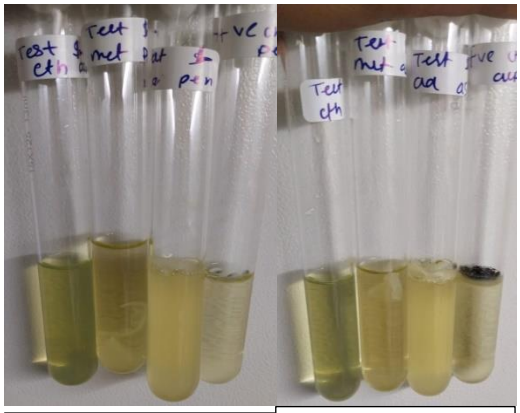
Table 6: List of Abbreviations.

Observation in table 4 indicates growth of all bacterial cultures in presence of methanolic and aqueous extracts. Various control tubes i.e Positive control, Negative control, Media control, Solvent control and Solvent with culture controls were maintained. Observations in table 5, shows growth in positive control which indicates that the bacterial cultures are viable and the media supports the growth of bacterial cultures. Solvent cultures controls also showed growth which indicates that the solvent had no effect on growth of bacterias confirming the sterility of media.

Antifungal Activity of leaf extracts:

Extracts	Cultures	Results (leaf)
Aqueous	***Aspergillus niger***	++
	Penicillium chrysogenum	++
Ethanolic	***Aspergillus niger***	-
	Penicillium chrysogenum	-
Methanolic	***Aspergillus niger***	+
	Penicillium chrysogenum	+

Table 7: Antifungal activity of alcoholic and aqueous extracts of leaf



Antifungal Activity against *Penicillium chrysogenum*

Antifungal Activity against *Aspergillus niger*

Extracts (Leaf)	**Controls**		
Ethanolic	**PC (RT)**	**NC (4^{O}C)**	**MC (RT)**
Results	+	-	-
	SOL (RT)	**SOL-Asp (RT)**	**SOL-Pen (RT)**
Results	-	+	+
Methanolic	**PC (RT)**	**NC (4^{O}C)**	**MC (RT)**
Results	+	-	-
	SOL (RT)	**SOL-Asp (RT)**	**SOL-Pen (RT)**
Results	-	+	+
Aqueous	**PC (RT)**	**NC (4^{O}C)**	**MC (RT)**
Results	+	-	-
	SOL (RT)	**SOL-Asp (RT)**	**SOL-Pen (RT)**
Results	-	+	+

Table 8: Activity of controls tubes maintained at various condition for antifungal activity.

Observation in table 7 indicates growth of fungal cultures in presence of leaves methanolic extract and excessive growth in presence of leaves aqueous extract. Fungal cultures were inhibited in presence of ethanolic extract of leaves. Observation table 8, indicates growth in positive control which indicates that the fungal cultures are viable and the media supports the growth of fungal cultures. Solvent cultures controls controls also showed growth which indicates that the solvent had no effect on growth of fungal cultures.

No growths of both the fungal cultures were observed in Negative control, Media control and Solvent control confirming the sterility of media.

CONCLUSION:

The above observed results and observations indicate that the shoot region i.e leaves and stems are rich in various secondary metabolites like flavonoids, alkaloids, terpenoids etc. The alcoholic extracts showed presence of more secondary metabolites as compared to aqueous extract. Antioxidants were produced more in stem as compared to leaves which was confirmed by determining antioxidant activity of the extracts. The ethanolic extract of leaves exhibited antibacterial activity against *Escherichia coli* and antifungal activity against *Penicillium chrysogenum* and *Aspergillus niger* which indicates that the weed plant *Tridax procumbens* is of great pharmaceutical and industrial importance.

REFERENCES:

1. Asmita Singh, Manisha Mathur, Ajay kumar,.Gajendra Pal Singh, Phytochemical screening and Thin layer chromatographic identification of Terpenoids from the root extract of Achyranthes aspera l.- An Indian Ethanomedicine, International Journal of Scientific and Research Publications, Volume 6, Issue 6, 2016

2. Aloisa Romero Camangian and Daisy Valdez Rivera, Phytochemical screening and biochemical analysis of selected herbal plants, Journal of critical reviews, volume 7, issue 11, 2020. 102-106.

3. Biren Shah, A. K. Sheth, Textbook of pharmacognosy and phytochemistry, General Methods for Extraction, Isolationand Identification of Herbal Drugs Elsevier, 407-452.

4. Mohammad Amzad Hossain, Khulood Ahmed Salim AL-Raqmi, Zawan Hamood AL-Mijizy, Afaf MohammedWeli, and Qasim Al-Riyami, Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris, Asian Pacific Journal of Tropical Biomedicine, 2013 Sep; 3(9): 705–710.

5. Santi Nur Handayani, Maylani Permata Saputri , Riani Utami and Jasmine Fadhila, Antibacterial Activity Test Ethanol Extract Leaf Ageratum conyzoides Linn against *Staphylococcus aureus* and *Escherichia coli* Bacteria, International Journal of ChemTech Research, volume 12 no. 5, 2019, 258- 262.

6. Suheda Koc, Belgin S. Isgor, Yasemin G. Isgor, Naznoosh Shomali Moghaddam & Ozlem Yildirim., The potential medicinal value of plants from Asteraceae family with antioxidant defense enzymes as biological targets, Pharmaceutical Biology, 2015; 53(5): 746–751.

7. Zeeshan M., Rizvi, S. M. D., Khan M. S. and Kumar A., Isolation, partial purification and evaluation of bioactive compounds from leaves of Ageratum houstonianum, EXCLI Journal 2012; 11: 78–88.