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Phytochemical and spectroscopic investigations *Tridax procumbens*

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Abstract
The present study was carried out to investigate the medicinally active substances present in the ethyl acetate leaf extract of *Tridax procumbens* (accession no: 3013/ Botany/St.Joseph college/Trichy) by using the analysis of TLC, UV-VIS, FTIR technique. The qualitative phytochemical analysis of powdered extracts show presence of phytoconstituents like flavonoids, saponins, cardiac glycoside, terpenoids, Quinones, alkaloids. The solvent system of TLC was Chloroform, ethanol, ethylacetate, hexane and acetic acid in the ratio of (10:2:5:1:1) was used and its *Rf* value was detected. From TLC analysis result, a spot was identified with R_f value was 0.82.The plant extract was scanned in the wave length ranging from 200-800 nm by using UV-Vis spectrophotometer and the characteristic peaks were detected. The plant extract UV spectrum observed a peak of 330nm.The FT-IR spectrum confirmed the presence of carboxylic acid, Alkane, ketone, Aldehyde, Amide, Aromatic, Haloalkane and Amine compounds in extract. The results shows that important bioactive compounds are present in plant extract and these constituents may be responsible for pharmacological activities.

Keywords: *Tridax procumbens*, plant extract, TLC, UV-VIS spectroscopy, FTIR Spectroscopy

Introduction

Taxonomic classification
Kingdom: Plantae
Subkingdom: Tracheobionta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Clade Angiosperms
Order: Asterales Clade Eudicots
Family: Asteraceae Tribe Heliantheae
Genus: *Tridax*
Species: *T. procumbens*
Binomial name: *Tridax procumbens*

Indian traditional medicinal system is fundamentally based on Ayurveda and there is an emerging interest of the world to study and to evaluate the rich heritage of traditional medicinal system and exploit the potential of natural bioactive components. From thousands of years ago the mankind has acquaintance about the benefits of different bioactive components with therapeutic potential. According to Ayurveda different plant extracts had significantly contributed for remedial effects on mankind. Even till today plant materials serves as the potential sources of drugs [1] This also indicates that the isolated compounds from herbal plants play an very important role in pharmaceutical industry, however, a lot of herbal plants still have not been explored for their phytochemical constituents [2, 3].Tridax procumbens L. belonging to family Asteraceae is a common medicinal herb used by ethno-medical practitioners. It is well known as a widespread weed and pest plant. It is native to tropical America but it has been spread to tropical, subtropical and mid temperate regions worldwide. The plant is a procumbent herb and is valued for its pharmaceutical properties [4]. It has been found to possess significant medicinal properties against stomach ache, diarrhea, dysentery, blood pressure, malaria, bronchial catarrh, wound healing, headache etc. It also prevents hair fall and check hemorrhage from bruises and cuts [5]. Its flowers and leaves possess insecticidal, antiseptic, and parasiticidal properties [6]. Its leaf extract is applied on the spot of cut to stop bleeding and pain. It has anticoagulant, antifungal, antidiarrhoel and insect repellent properties [7-8]. It has hepatoprotective activity [9], anti-inflammatory [10], and wound healing [11, 12] antidiabetic activity [13] hypotensive effect, immunomodulating property [14], anticancer activity [15], and antioxidant activity [16-18].

Materials and Method

Collection of plant

Leaves of *Tridax procumbens*was collected and washed thoroughly in water to remove mud and dust particles. The leaves were shade dried and then powdered coarsely in mixer and stored in separate air tight containers at room temperature for further use.

Preparation of plant extract

10 g of fine powdered sample was taken and mixed with 200 ml ethylaetate. Samples were initially soaked in respective solvent for 24 h under refrigeration and subjected to ultra sonication at 450Hz for 5 cycle. Ultrasound assistant extraction was carried out using Bandelin Sonorex brand ultrasonic bath.

Phytochemical Analysis

Test for carbohydrates

To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish colour indicates the presence of carbohydrates.

Test for phenols and tannins

Crude extract was mixed with 2 ml of 5% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for flavonoids (Shinoda test)
One to five drops of concentrated hydrochloric acid (HCl) were added to little amount of ethanolic extract of the plant material. Immediate development of a red colour indicates the presence of flavonoids.

Test for saponins
Extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Glycosides
Salkowski's test
Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

Keller-kilani test
Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.

Test for terpenoids
Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish color indicated the presence of terpenoids.

Test for quinones
A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

Test for alkaloids
Two mL of extract was taken in a test tube and then 0.2 mL dilute HCl was included, followed by 1 mL of Meyer's reagent. A yellowish coloration indicates alkaloid's presence.

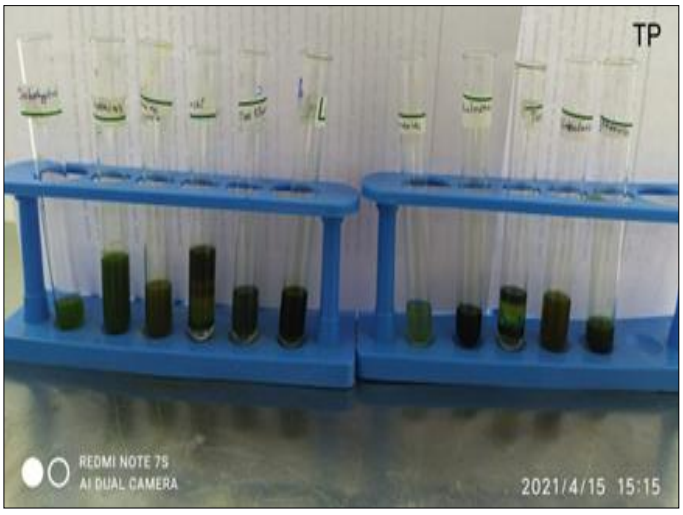
Test for phlobatannins
To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of phlobatannins.

TLC-Thin layer chromatography
Samples were eulted with column using silica gel 120 mesh with Chloroform: ethylacetate (10:2) and TLC performed on precoated TLC plates (silica gel 60F-254). Chloroform-ethanol-ethylacetate-hexane and acetic acid (10:2:5:1:1) is used as mobile phase. Samples were placed on TLC using capillary tube at the bottom of plate and allowed to dry. The plate is kept under mobile phase and separation of compounds permitted until the solvent reached ¾ the distance and exposed under UV at 365 and 254 nm.

UV
The ethyl acetate plant extract was examined under UV-Visible spectral analysis.The sample was diluted to 1:10 with the same solvent. The extract was scanned in the wavelength ranging from 200-800nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected.

FTIR
FTIR analysis of the aqueous extract was carried out through the potassium bromide (KBr) pellet (FTIR grade) method in 400-4000 cm^{-1} and spectrum was recorded using Jasco FT/IR-6300 Fourier transform infrared spectrometer equipped with JASCO IRT-7000 Intron Infrared Microscope using transmittance mode operating at a-1 resolution of 4 cm (JASCO, Tokyo, Japan).

Result and Discussion
Phytochemical test
The qualitative phytochemical analysis of *Tridax procumbens* (plate 1a) was summarized in table1. Flavonoids, Saponins, cardiac glycoside, Terpenoids, Quinones, Alkaloids were present in ethyl acetate extract of *Tridax procumbens* and Carbohydrate, Phenols, Tannins, Phlobatannins are absents of ethyl acetate extract for *Tridax procumbens*. Thin layer chromatogram of ethyl acetate extract of *Tridax procumbens* was given in plate 1b and TLC of ethyl acetate extract of *Tridax procumbents* revealed the presence of a spot having R_f value of 0.82 when a solvent phase of Chloroform, ethanol, ethylacetate, hexane and acetic acid in the ratio of (10:2:5:1:1) a solvent system was used.



a Phytochemical test


1b.TLC.

Plate 1

Spectral characterization

UV-Vis spectrophotometer is related to the spectroscopy of photons in the UV-visible region. UV-Vis spectroscopy uses light in the visible ranges or its adjacent ranges. The color of the chemicals involved directly affects the absorption in the visible ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic spectrum[19]. In the present study UV-Vis spectral profile showed the peaks 330nm figure-1.

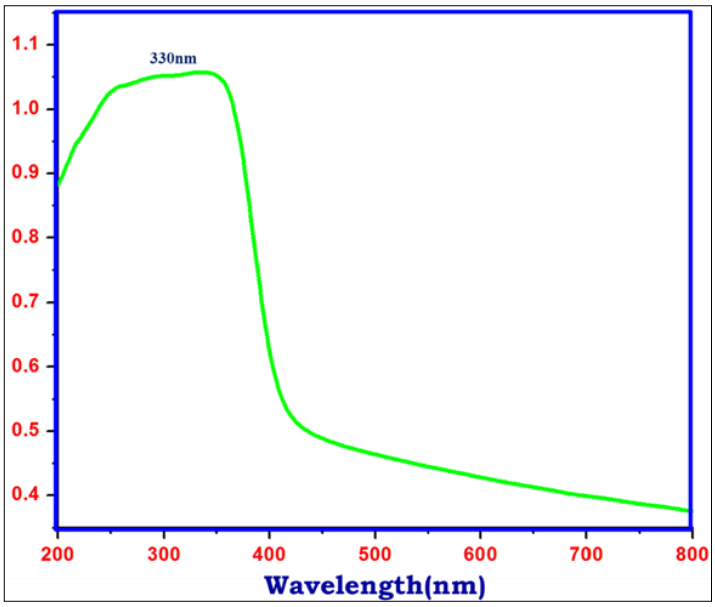


Fig 1: UV analysis of *Tridax procumbens*

FTIR

The FT-IR has proven to be a valuable tool for characterization and identification of compounds or functional groups (chemical bonds) present [20, 21]. It allows the qualitative determination of organic compounds as the appearance of the bands in the infrared spectrum at a specific frequency, which is further influenced by the surrounding functional groups [22]. The results of FTIR peak values and functional groups were represented in table 2 and figure 2. When the plant extract was passed into the FTIR spectrum, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of functional groups such as carboxylic acid (3333.89), Alkane (2919.00, 2109.96, 2054.48), Aldehyde (2850.71, 1604.43, 1157.60) Ketone (1718.46), Amide (1636.89), Aromatic (1448.35), Haloalkane (1376.51, 1279.28, 1242.91, 1101.60, 899.90, 861.49, 740.52, 656.88), Amine (1199.94, 1071.07, 1033.33).

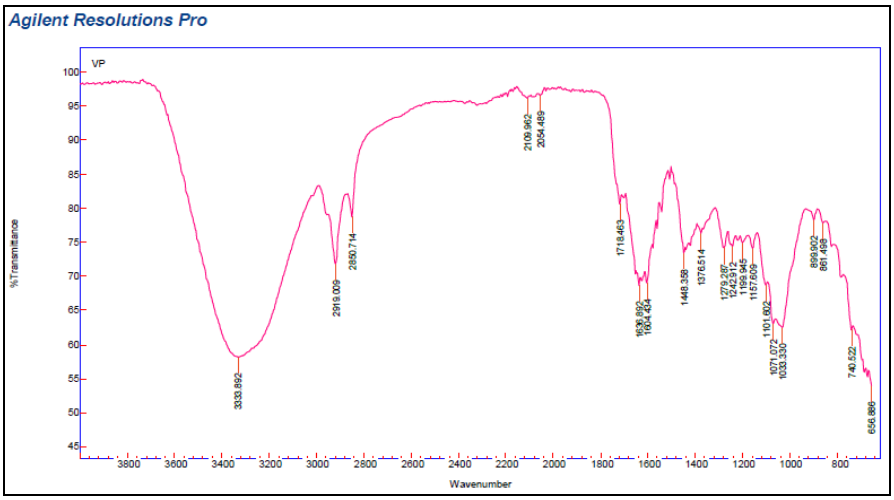


Fig 2: FTIR analysis of *Tridax procumbens*

Table 1: Phytochemical test for *Tridax procumbens*

S.no	Phytochemical test	Results
1.	Carbohydrate test	-
2.	Phenols test	-
3.	Tannins test	-
4.	Flavonoids test	+
5.	Saponins test	+

6.	Cardiac glycoside	
	1. Salkowski's test-	+
	2. keller- kilani test-	+
7.	Terpenoids test	+
8.	Quinones test	+
9.	Alkaloids test	+
10.	Phlobatannins test	-

Table 2: FT- IR spectral peak values and functional groups obtained for Tridax procumbent

S.no	Peak value (cm^{-1})	Strech	Functional group
1	3333.89	O-H	Carboxylic acid
2	2919.00	C-H	Alkane
3	2850.71	C-HO	Aldehyde
4	2109.96	C-H	Alkane
5	2054.48	C-H	Alkane
6	1718.46	C=O	ketone
7	1636.89	C=O	Amide
8	1604.43	C=O	Aldehyde
9	1448.35	C=C	Aromatic carbon
10	1376.51	C-F	Haloalkane
11	1279.28	C-F	Haloalkane
12	1242.91	C-F	Halo alkane
13	1199.94	C-N	Amine
14	1157.60	C=O	Aldehyde
15	1101.60	C-F	Haloalkane
16	1071.07	C-N	Amine
17	1033.33	C-N	Amine
18	899.90	C-Cl	Halo alkane
19	861.49	C-Cl	Halo alkane
20	740.52	C-Cl	Halo alkane
21	656.886	C-Cl	Halo alkane

Conclusion

In this study we conclude that the presence of phytochemical study of Flavonoids, Saponins, cardiac glycoside, Terpenoids, Quinones, Alkaloids.FTIR analysis were performed and the results revealed the presence of compounds carboxylic acid, Alkane, Aldehyde, ketone,Amide, Aromatic, Haloalkaneand Amine compounds in extracts. Hence these results clearly indicate that this plant can be used for the further development of phytomedicines for medicinal purposes.

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