

PHYTOCHEMICAL STUDIES OF THE LEAVES OF TRIDAX PROCUMBENS

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ABSTRACT

Tridax procumbens is a common medicinal herb which is best known as a widespread weed and pest plant distributed throughout India. The chemical constituents of the plant showed that its leaves contain various alkaloids, flavonoids, carotenoids, fumaric acid etc. Their extract possesses various pharmacological properties (anti-inflammatory, hepatoprotective, immunomodulatory, antimicrobial or antibacterial, activity antiseptic, anti-cancerous activity, repellency activity, hemostatic activity, antidiabetic, anti-urolithiatic activity, hypotensive, antioxidant effect, bradycardiac effects etc.). A number of researchers are working on its active constituents in various fields to develop it as a natural ayurvedic medicine against various ailments and disorders. This research work has been undertaken to extract any new or known chemical constituent of *Tridax Procubens*.

Keywords:

Introduction

Tridax procumbens is a species of flowering plant in the daisy (*Asteraceae*) family. It is best known as a widespread weed and pest plant. The plant is native of tropical America and naturalized in tropical Africa, Asia, Australia. It is a wild herb distributed throughout India. It is annual or biennial somewhat patently hispid herbs. Stem branched, creeping at base, sub erect or trailing above^[1].

Leaves ovate-lenseolate, or elliptic- rhomboid, with a cineaste base, obtuse or sub acute, coarsely serrate or lobed, 2.5-7 cm long. Heads solitary, 1.2-1.5 cm across, on erect, 10-30 cm long peduncle. Marginal flowers 5-6 with pale yellow, 0.3 cm long ligules; disc flowers bright yellow^[2-3]. *Tridax procumbens* is found to possess pharmacological activities like hepatoprotective effect, immunomodulating property, promising wound healing activity, antidiabetic, hypertensive effect, antimicrobial, insect repellent activity, anti inflammatory and antioxidant, bronchial catarrh, dysentery, diarrhoea also prevent falling of hairs and leads to hair growth promotion^[4]. Its common names include coat buttons and tridax daisy in English, Jayanthi in Kannada, cadillo chisaca in Spanish, herbe caille in French, Jayanti veda in Sanskrit, ghamra in Hindi, Bishalya karani in Oriya, Kambarmodi in Marathi, Gaddi Chemanthi in Telugu, vettukaaya poondu in Tamil, and kotobukigiku in Japanese.^[5]

Classifications of *Tridax procumbens* :^[5]

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliophyta
Subclass	Asteridae
Order	Asterales
Family	Asteraceae
Genus	<i>Tridax</i> L.
Species	<i>Tridax procumbens</i>

List of various Pharmacological activity of parts of plant *Tridax procumbens*

Whole plant - Antimicrobial activity against both gram-positive and gram-negative bacteria, Anti coagulant, Anti inflammatory.

Flowers leaves - Anti septic, Insecticidal, Parasiticial.

Aerial parts – Hepatoprotective .

Leaves - Wound healing, To check haemorrhage from cuts, bruise and wounds, Hypertensive activity, Antidiabetic activity, Dysentery, Diarrhoea, To prevent falling of hair and promotes the growth of hair, bronchial catarrh treatment, against conjunctivitis, immunomodulating property, insect repellent activity^[3].

Objectives

- To extract the leaves of *Tridax procumbens* by using suitable solvent (ethanol).
- To separate out the chemical compound from the crude extract of the leaves of *Tridax procumbens* by the help of suitable chemical separation
- To analysis chemical group present in the crude extract in the leaves of the *Tridax procumbens* to get preliminary idea about the compound present in the extract by the help of chemical analysis and spectroscopic analysis.

Material and Methods

Leaves of *Tridax Procumbens* :

First we identified and collect the plant leaves from the place of Dundukhera, Distt Shamli, Uttar Pradesh, India. After the collection of leaves, the leaves dried in the sun light and extracted in soxhlet in the solvent of ethanol and separate the leaves crude by distillation method. In the leaves crude we add hexane and separate out solution of hexane soluble compound. We separate out the all compound by column chromatography and characterize by the help of spectroscopic technique.

Apparatus and chemicals used

Soxhlet extractor, forceps clamp stand, heating mantle, three round bottomed flask, reflux condenser, heating mantle and chiller, TLC plate, column for chromatography, distillation apparatus iodine chamber and test tubes.

Ethanol, Hexane, Ethyl acetate, Silica gel, Pet ether and distilled water.

Extraction

The extraction of the plant material can be obtained by drying the of the given plant under the shade and is grinded to form a fine powder. The dried powdered material then obtained is percolated with polar solvent hexane for 48 hours in soxhlet extractor followed by non polar solvents hexane.

Procedure of Extraction

- 124 gm. Powdered form of *Tridax procumbens* is poured into soxhlet extractor and its level is maintained.
- One liter of Ethanol is being poured into 5000ml round bottomed flask. Vacuum grease is being used on movable parts to avoid friction.
- Also cotton and Aluminum foil is being used to cover connectors to prevent oozing.
- After setting the soxhlet extractor electricity is being supplied through heating mantle and temperature is maintained at 60°C.
- Then soxhlet extractor is being connected to chiller to provide chilling.
- This process is continued for 48 hours.
- After that Ethanol extract obtained is put to distillation to separate the crude extraction.
- We found the 22.5 gm. Leaves crude by the help of ethanol.
- Add Hexane in crude and separate out the soluble compound and checked by TLC. The total weight of hexane separated crude is 10 gm.

Separation of Chemical constituents

First we take the hexane solution of the crude and add silica gel (20 mesh size) in solution and make fine powder. Take a column and pack with silica gel and add this mixture in top of column, add hexane and run the column. The compound are separate out and checked with TLC and collect in a tube. The compound getting after crystallization is taken for column chromatography and column is used for this process. Silica gel 60-120 and hexane is used for packing the column. After packing, pour the given compound into the column. Firstly add 100 ml hexane and followed by 90ml hexane and 10 ml ethyl acetate, 80ml hexane and 20ml ethyl acetate is added. Each compound separated is then taken for thin layer chromatography.

The Adsorbent

Silica gel (SiO₂) 60-120 is used for column chromatography. This number refers to the mesh of the sieve used to size the silica, specifically, the number of holes in the mesh or sieve through which the crude silica particle mixture is passed in the manufacturing process. If there are more holes per unit area, those holes are smaller, thus allowing only smaller silica particles go through the sieve. The relationship is: the larger the mesh size, the smaller the adsorbent particles.

Analysis of Column Eluents

If the compounds separated in a column chromatography procedure are colored, the progress of the separation can simply be monitored visually. More commonly, the compounds to be isolated from column

chromatography are colourless. In this case, small fractions of the eluent are collected sequentially in labelled tubes and the composition of each fraction is analyzed by thin layer chromatography. The slurry (extract +silica gel) of different extracts of different plant materials is loaded in a column and different solvents (hexane ,chloroform and methanol) are used to isolate the pure compounds .The collected fractions are concentrated and then detecting the spot by thin layer chromatography.

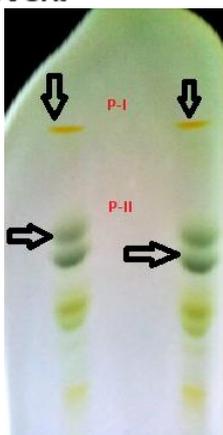


Fig 3: separation of the compound in column chromatography

Thin layer chromatography

Thin layer chromatography is done as using a thin, uniform layer of silica gel coated onto a piece of glass. The silica gel is the stationary phase. The stationary phase for thin layer chromatography also often contains a substance which fluoresces in UV light. The mobile phase is a suitable liquid solvent or mixture of solvents. The R_f value for each compound is then worked out using the formula

$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$



Crystallization

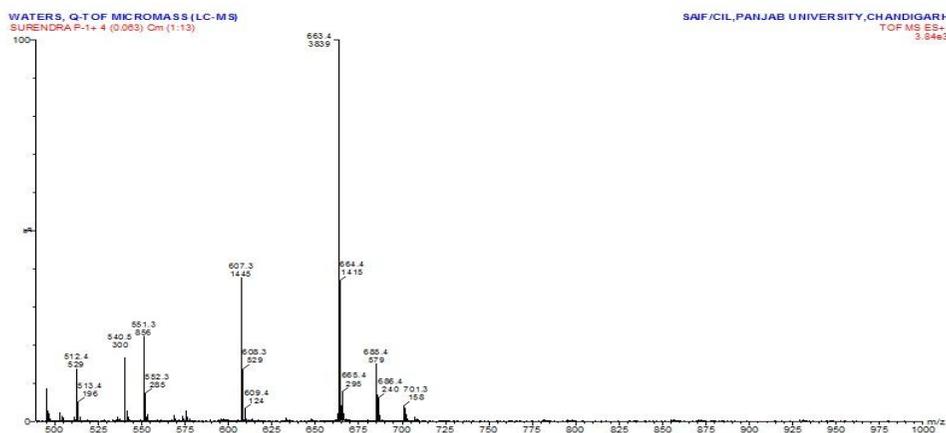
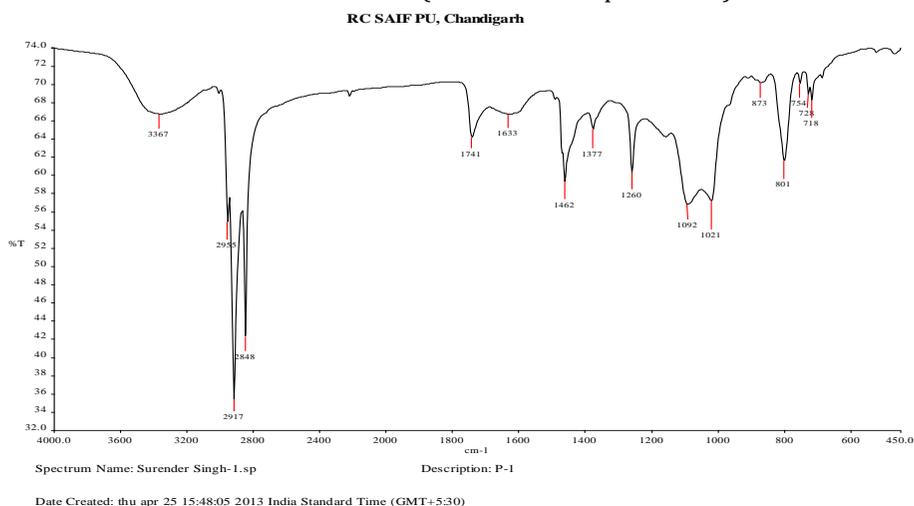
Take Hexane extract and dissolve it in 50ml hexane .After the solution becomes clear then keep the Hexane extract in beaker and cover the beaker with aluminum foil and make small pores on aluminum foil so that solvent gets evaporated leaving behind the compound keep it in dark.

Spectroscopic analysis

After the crystallization of separated compound the compound are seal in a glass tube and send to Punjab University, Chandigarh for characterization of spectroscopic analysis as NMR spectroscopy, Mass Spectroscopy, Infrared Spectroscopy C^{13} spectroscopy and UV Spectroscopy.

✓ The first compound are separated with hexane and spectral analyse data are given below

1. FTIR result of P-1 (Pure Hexane separation 1)



2. Mass Spectra of P-1 (Pure Hexane separation 1)

Results and Discussion**About P-I Sample**

The first compound separated by hexane in column chromatography and recrystallized with methanol checked by TLC. The first compound is yellow in colour and this compound is **3, 6-Dimethoxy-5,7,2,3,4-pentahydroxyflavone 7-O-D-glucopyranoside** and physical state of this compound is brownish yellow crystalline powder and the odour of the compound is like sweet mango. Melting point of this compound is 234°C. In Thin layer chromatography the R_f value is 1.09 (in Hexane). This compound is Practically insoluble in water and soluble in Hexane and Methanol. Molecular weight of this compound is 540 g mol⁻¹ and molecular formula is C₂₃H₂₄O₁₅.

Characterization

NMR Result: (300 MHz, DMSO): sigma 7.52 (1H, d, J 9.24 Hz H-5'), 7.08 (1H, d, J 9.24 Hz, H-6'), 6.76 (1H, s, H-8), 5.10 (1H, d, J 7.25 Hz, H-1'), 3.91 (1H, m, H-2'), 3.8 (3H, br s, OMe), 3.72 (1H, m, H-4'), 3.71 (3H, br s, OMe), 3.69 (1H, m, H-2'), 3.59 (3H, m, H-5'), 3.46 (1H, d, J 8.02 Hz, H-6'a), 3.30 (1H, d J 6.76 Hz, H-6'b).

Inferred result: 3367, 2955, 2917, 2848, 1741, 1633, 1462, 1377, 1260, 1092, 1021, 873, 801, 754, 728, 718 cm⁻¹.

C¹³NMR: (75 MHz, DMSO): 151.7 (C-2), 138.5 (C-3), 179 (C-4), 156.40 (C-5), 121 (C-6) 156 (C-7), 94.5 (C-8), 152.4 (C-9), 106.6 (C-10), 122.50 (C-11), 132.6 (C-12), 146.7 (C-13), 150.8 (C-14), 116 (C-15), 112.4 (C-16), 101 (C-17), 74 (C-18), 77.02 (C-19), 70 (C-20), .

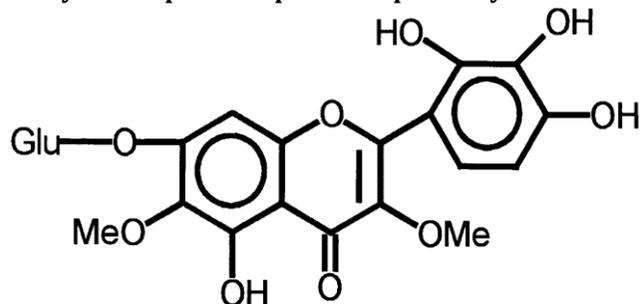
Mass spectroscopy: 344 (2.1), 329 (2.1), 286 (4.4), 1.94 (3.1), 181 (131.3), 180 (25.5), 177 (9.2), 165 (9.4), 163 (20.1), 151 (10.7), 150 (10.7), 148 (15.1), 146 (8.9), 137 (20.5), 124 (19.7), 132 (18.8), 122 (60.3), 120 (23.5) 69 (100).

UV result: (MeOH): 253(log e 4.1), 271(4.3), 328(3.1); (+AlCl₃): 273, 371; (+NaOAc + H₃BO₃): 273, 343 nm.

Conclusion

Based upon spectroscopic analysis and melting point of separated chemical compound of *Tridax Procumbens*, it can be concluded that this compound is already known compound ^[16] and is **3, 6-Dimethoxy-5,7,2',3',4'-pentahydroxyflavone 7-O-D-glucopyranoside**.

Structure of the compound by the help of the spectroscopic analysis



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