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PHYTOCHEMICAL SCREENING AND GC-MS STUDIES OF THE METHANOLIC EXTRACT OF *TRIDAX PROCUMBENS*

Pankaj Kushwaha, Shiv Shankar Yadav, Vigyan Singh and L. K. Dwivedi *

Institute of Biomedical Sciences, Bundelkhand University, Jhansi - 284128, Uttar Pradesh, India.

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Correspondence to Author:
Dr. Lavkush Dwivedi

Assistant Professor,
Institute of Biomedical Sciences,
Bundelkhand University, Jhansi - 284128, Uttar Pradesh, India.

E-mail: lavkush@bujhansi.ac.in

ABSTRACT: Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. *Tridax procumben*s Linn. (Asteraceae) one of the medicinally important plants commonly found in subtropical countries growing primarily at waste places, roadsides throughout India during the raining season was chromatographically evaluated in the present work for various phytochemicals found in it. The phytochemical tests showed the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins in methanolic extract of *Tridax procumbens* (METP). Majorly 25 compounds found in 82.81% peak area were identified through spectrum matching with National Institute Standard and Technology (NIST) database. As per known effects of the observed compounds like octadecanoic acid, 11, 14, 17- Eicosatrienoic acid, methyl ester, 9, 12, 15-Octa-decatrienoic acid, methyl ester (Z,Z,Z,)-, phthalic acid, 6-Methylhept-2-Yl-Tridecyl ester the *T. procumbens* reflected some novel therapeutic effects like anti-microbial, anti-cancer, anti-hair fall, CNS depressant, analgesic, anti-inflammatory, antipyretic, anti-arthritic, anti-coronary, anti-neoplastic, immunosuppressive, anti-spermatogenic activity. Furthermore, identification and purification of the active compounds responsible for the therapeutic activity may prove the plant of great pharmacological importance.

INTRODUCTION: Medicinal plants being rich in numerous active constitutes of therapeutic value are used as a commendable source of remedy for treating human diseases [1]. The irreversible effects of modern therapies and increasing drug resistance have augmented our reliance on medicinal plants for a herbal remedy against the deadly and infectious diseases [2]. Today, about 40 percent of population reporting use of the herb to treat medical illness [3]. *Tridax procumbens* commonly known as Coat Button and Ghamra is a most potent species of widespread weed and flowering plant among 30 species belonging to family Asteraceae.

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It is native of tropical America, naturalized in tropical Africa, Asia, and Australia. In India, it is found in the almost the whole part of the country. The various parts of the plant have been reported for their therapeutic effects against dysentery, epilepsy, hypertension, hemorrhage and metabolic syndrome [4]. Traditionally, *T. procumbens* has been known for its anti-coagulant, anti-fungal, and insect repellent activity [5]. Its use as bio-adsorbent for the removal of Cr (VI) from industrial wastewater has made it a plant of industrial importance.

Moreover, hepatotoxicity [6], antioxidant [7] and hair growth promoting activity [8] are widely reported activity of the plant. By the existing reports, *T. procumbens* have been one of the important medicinal plants being used for its ant-ailments effects from the old days to the present. To explore more pharmacological properties of the plant a phytochemical screening and GC-MS based compound analysis of the methanolic extract of leaves was done in the present study.

MATERIALS AND METHODS:

Collection of Plants: The leaves of *T. procumbens* (Linn.) were collected from, different places, such as Bundelkhand University Campus Jhansi, Baragaon Jhansi, Uttar Pradesh, and Forest Nursery of Bhagwantpura, Orchha (Madhya Pradesh) after sample verification (No. 28153) from the CCRAS-Regional Ayurveda Research Institute, Ministry of AYUSH, Govt. of India Gwalior Road, Jhansi-284003 in April 2018. *T. procumbens* was washed and shade dried for 2 weeks. After drying, the homogenate was transformed into a fine powder by using an electric mixer.

Preparation of Plant Extract: A portion of dried leaves (100 g) of *Tridax procumbens* was placed in a Soxhlet apparatus. Extraction was performed with 500 ml of methanol for 24 h at 64 °C. The extract was filtered through a Whatmann filter paper no. 41 (110 mm). The resulting solution was concentrated in vacuum to give dryness to the methanol extract. The extract was stored in a refrigerator at 4 °C for further use.

Preliminary Phytochemical Screening: Preliminary phytochemical screening and quantitative test for the presence of phenols, tannins, flavonoids, alkaloids, terpenoids, anthraquinones, steroid, and saponins was carried out using standard test protocols [9]. These phytochemicals were identified by characteristics color change using standard procedures [10].

Tests for Phenols:

Phenols Test: The formation of intense color on the addition of 0.5 ml of $FeCl_3$ (w/v) solution into 2 ml of test solution indicated the presence of phenols [11].

Test for Flavonoids:

NaOH Test: 2-3 ml of extract and few drops of sodium hydroxide solution were added into a test tube. Formation of the intense yellow color that didn't become colorless on the addition of a few drops of dilute HCl indicated the absence of flavonoids [12].

Shinoda Test: 2-3 ml of extract and few fragments of magnesium metal were added into a test tube, followed by dropwise addition of concentrated HCl. Formation of magenta color indicated the presence of flavonoids [13].

Test for Tannins:

Gelatin Test: Gelatin (gelatin dissolves in warm water immediately) solution was added into the extract. Formation of white precipitate indicated the presence of tannins [14].

Lead Acetate Test: Few drops of 10% lead acetate solution were added into 5 ml of extract. Formation of yellow or red precipitate indicated the presence of tannins [14].

Test for Saponins:

Foam Test: The extract was diluted into 20 ml of distilled water and shaken in a graduated cylinder for 15 min. A 1 cm layer of foam indicated the presence of saponins [13].

Haemolysis Test: One drop of extract and one drop of blood was placed on the glass slide. Haemolytic zone appeared [13].

Test for Alkaloids:

Iodine Test: Addition of a few drops of dilute iodine solution into 3 ml test solution resulted in blue color which disappeared on boiling and reappeared on cooling [12].

Wagner's Test: Few drops of Wagner's reagent were added into 2 to 3 ml in the extract. Formation of reddish brown precipitate indicated the presence of alkaloids [15].

Test for Steroids: Acetic anhydride (2 ml) was added to 0.5 g of methanol extract of each sample with 2 ml sulphuric acid. A color change from violet to blue or green in some samples was an indication of the presence of steroids.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis: GC-MS analysis was carried out on GC Clarus 500 Perkin Elmer system comprising an AOC-20i autosampler and Gas Chromatograph interfaced to a Mass Spectrophotometer (GC-MS) to study the phytochemical components present in the extract. The instrument was employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm i.d × 1 EM df, composed of 100% dimethyl polysiloxane), operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min, and an injection volume of 0.5 EI was employed (split ratio of 10: 1 injector

temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min). With an increase of 10 ºC/min, to 200 ºC then 5 ºC/ min to 280 ºC, ending with a 9 min isothermal at 280 ºC. The GC-MS was run for 30 min, and Mass spectra were taken at 70eV; a scanning interval of 0.5 and fragments from 40 to 550 Da.

Identification of Components: The Mass Spectrum of GC - MS was interpreted using National Institute Standard and Technology (NIST) database having more than 79,000 patterns. As a consequence, name, molecular weight, and structure of the components of the test materials were ascertained by comparing the spectrum of the unknown component with the spectrum of the known components stored in the NIST library.

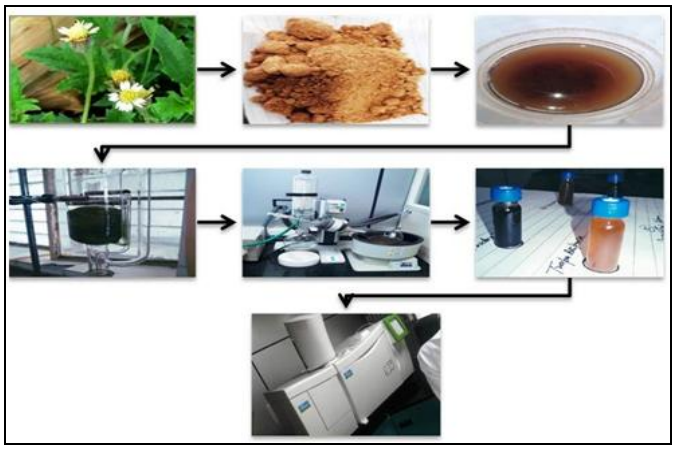

FIG. 1: SHOWING STEPS OF THE WORK

RESULTS:

Preliminary Phytochemical Screening and Quantitative Test: After the successful conventional hot Soxhlet extraction of the *Tridax procumbens* leaves, the preliminary phytochemical study revealed that methanolic extract of *Tridax procumbens* Linn. contains alkaloids, flavonoids, phenols, saponins, steroids, and tannins. The list is summarized in **Table 1**.

More than 20 peaks were generated in the GC-MS spectrum of methanolic extract of *T. procumbens* (METP) showed in **Fig. 2**. Furthermore, the Mass Spectra of identified compounds from METP were matched with NIST/NBS spectral database. The list of identified compounds covering 82.81% peak area are given in **Table 2** with their retention time, molecular formula, molecular weight, and reported biological activity.

TABLE 1: PRELIMINARY PHYTOCHEMICAL EVALUATION OF METHANOL EXTRACTS OF *TRIDAX PROCUMBENS*

Phytochemical constituents	Test	Result
Phenol	Phenol	(+)
Flavonoids	Shinoda	(+)
	NaOH	(−)
Tannins	Lead acetate	(+)
	Gelatin	(+)
Saponins	Foam	(+)
	Hemolysis	(+)
Alkaloids	Iodine	(+)
	Wagner's test	(−)
Steroid	Acetic anhydride test	(+)

(+) = Present; (-) = Absent

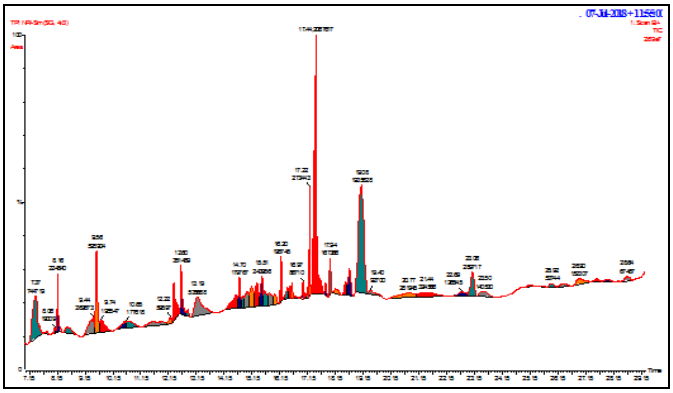

FIG. 2: GC-MS SPECTRUM OF METHANOLIC EXTRACT OF *TRIDAX PROCUMBENS*

From the analysis, 25 compounds were elucidated from 82.81% peak area (given in **Table 2**). The major components present at RT 17.44 (peak area 22.83%), RT 19.05 (peak area 21.38%) were identified as Tetradecanoic acid, Dodecanoic acid, *n*-Hexadecanoic acid, Eicosanoic acid, Octadecanoic acid, Tridecanoic acid, Pentadecanoic acid, 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z)-, 11, 14, 17-Eicosatrienoic acid, methyl ester and 9, 12, 15-Octa-decatrienoic acid, methyl ester (Z,Z,Z,)-. Moreover, 4-Octanol (RT 7.42; peak area 8.22%), 2a, 3, 4, 5-Tetrahydrobenz(Cd) Indole-2(1h)-One (RT 13.18; peak area 5.91%), Dodecane, 1-Chloro- (RT 12.45; peak area 3.88%), Phthalic acid, 6-Methylhept-2-Yl-Tridecyl ester (RT 23.65; peak area 3.19%) were identified. Except this several other compounds covering <3% peak area % were also noted.

DISCUSSION: The GC/MS analysis showed 8-9 compounds majorly found in the extract which explain the therapeutic potential of the plant. The pharmacological activity of highest found

compounds like Octadecanoic acid (RT 17.44; peak area 22.83%), 11,14,17-Eicosatrienoic acid, methyl ester (RT 19.05; peak area 21.38%), 9, 12, 15-Octadecatrienoic acid, methyl ester (Z,Z,Z,)- (RT 19.05; peak area 21.38%), Phthalic acid, 6-Methylhept-2-Yl-Tridecyl ester (RT 23.65; peak area 3.19) proved it the plant of great pharmacological importance. As the reported compounds are known for their very good therapeutic effects *viz.*, antimicrobial [16, 17], anti-inflammatory [18], gene regulation activity, anti-arthritic [19], anti-coronary, CNS depressant, anti-neoplastic, anti-spermatogenic and immuno-suppressive activity (detailed in **Table 2**).

TABLE 2: DETAIL OF COMPOUNDS IDENTIFIED FROM GC-MS ANALYSIS OF METHANOLIC EXTRACT OF *TRIDAX PROCUMBENS*

S. no.	RT	Compound name	%age Peak area	Mol. Formula	Mol. Weight	Biological activities
1	7.42	4-Octanol	8.22	$C_8H_{18}O$	130	Used in the treatment of essential tremor
2	8.16	1-Napthalenyl dodecanoate	2.48	$C_{22}H_{30}O_2$	326	Used in the study of progestin distribution and progestin tissue receptor
3	8.48	Benzaldehyde,4 -Methyl	2.90	C_8H_8O	120	Flavoring ingredient
4	9.44	Benzofuran,2,3-Dihydro	2.19	C_8H_8O	120	Analgesic and anti-inflammatory
5	10.65	Fosfosal	1.96	$C_7H_7O_6P$	218	Analgesic and anti-inflammatory
6	10.65	Aspirin	1.96	$C_9H_8O_4$	180	Analgesic and anti-inflammatory, antipyretic
7	10.65	Salicylic acid	1.96	$C_7H_6O_3$	138	Bacteriostatic, fungicidal, keratolytic, analgesic
8	12.45	Dodecane, 1-Chloro-	3.88	$C_{12}H_{25}Cl$	204	-
9	13.18	2a,3,4,5-Tetrahydrobenz (Cd)Indole-2(1h)-One	5.91	$C_{11}H_{11}ON$	173	Serotonin and dopamine receptor
10	15.50	Undecanoic acid	2.69	$C_{11}H_{22}O_2$	186	Flavoring agent
11	16.20	Phytol acetate	2.16	$C_{22}H_{42}O_2$	338	Food additive
12	17.22	Benzene propanoic acid, 3,5-Bis(1,1-Dimethylethyl)-4-Hydroxy-, Met	3.02	$C_{18}H_{28}O_3$	292	Intermediates, the oxidizing /reducing agent
13	17.44	Tetradecanoic acid	22.83	$C_{14}H_{28}O_2$	228	Used to synthesize flavor and as an ingredient in soaps and cosmetics
14	17.44	Dodecanoic acid	22.83	$C_{12}H_{24}O_2$	200	Antimicrobial
15	17.44	N-Hexadecanoic acid	22.83	$C_{16}H_{32}O_2$	256	Used in determination of water hardness. It is also used in echo enhancement in sonographic doppler B-mode imaging
16	17.44	Eicosanoic acid	22.83	$C_{20}H_{40}O_2$	312	Surfactants
17	17.44	Octadecanoic acid	22.83	$C_{18}H_{36}O_2$	284	Antimicrobial, anti-inflammatory
18	17.44	Tridecanoic acid	22.83	$C_{13}H_{26}O_2$	214	Surfactants
19	17.44	Pentadecanoic acid	22.83	$C_{15}H_{30}O_2$	242	Good biological marker
20	19.05	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	21.38	$C_{18}H_{30}O_2$	278	Gene regulation activity
21	19.05	11,14,17-Ecosatrinoic acid, methyl ester	21.38	$C_{21}H_{36}O_2$	320	Antiarthritic, anti-coronary anti-inflammatory
22	19.05	9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z,)-	21.38	$C_{20}H_{34}O_2$	292	Anti-inflammatory and CNS depressant
23	23.65	Bis(2-Ethylhexyl) phthalate	3.19	$C_{24}H_{38}O_4$	390	Plasticizers, phthalates food contaminant
24	23.65	Phthalic acid, 2-Ethylhexyl neopentyl ester	3.19	$C_{21}H_{32}O_4$	348	Potential, biomarker
25	23.65	Phthalic acid, 6-Methylhept-2-Yl-Tridecyl ester	3.19	$C_{29}H_{48}O_4$	460	Anti-neoplastic, immunosuppressive

CONCLUSION: The presence of various bioactive compounds in the methanolic extract of *Tridax procumbens* justifies that the aerial part of the plant has considerable potentials. They can

further be explored for their optimum use in the treatment of various ailments like infectious diseases, inflammatory diseases, arthritis, coronary diseases, cancer, neurological disorders, and immuno-modulation. Moreover, analgesic, antipyretic and gene regulatory activities of the identified compounds may also be a point of significant interest.

However, isolation of individual phytochemical constituent and further study of its biological activity will give more fruitful results. Hence, further research is necessary to identify and purify the active compounds responsible for the therapeutic activity.

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CONFLICT OF INTEREST: There are no conflicts of Interest.

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