ORIGINAL ARTICLE

Metabolite profiling and antioxidant activity of leaves, stem, and flowers of *Tridax procumbens*: A medicinal herb

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Abstract

Introduction: This study was designed to analyze *in vitro* antioxidant activity and estimate the metabolic profiling of non-polar and polar extracts of *Tridax procumbens* leaves stem and flowers. **Materials and Methods:** Antioxidant activity of hexane and methanol extract was determined through DPPH free-radical scavenging assay and nitric oxide assay using the standard method and metabolite profiling of non-polar and polar extracts of *T. procumbens* was done by using gas chromatography-Mass spectrometry (GC-MS). **Results:** The hexane extract of *T. procumbens* flower (HTPF) showed the most significant antioxidant activity concerning the methanolic extract, which is indicated by its IC_{50} values obtained from the DPPH was 33.14 µg/mL and 34.52 µg/mL (HTPF and MTPL) wherein IC_{50} for nitric oxide assay was 16.74 and 34.26 µg/mL (HTPF and MTPL), respectively. In addition, GC-MS-based metabolic profiling of *T. procumbens* parts (leaves, stem, and flowers) showed hundred and more chemically diverse metabolites. A very high concentration of oleanolic acid was found in hexane leaves (32.13 ± 0.3.11%) and flowers (17.66 ± 1.71%) extract while in stems (12.63 ± 1.22). The other metabolites such as 2, 4-di-*tert*-butyl phenol, and germacrene were identified as major metabolites. *Trans*-farnesol and two major fatty acids palmitic and linolenic and high percent peak area of stearic acid were detected in stems. **Discussion and Conclusion:** GC-MS analysis showed several compounds with, high content of terpenes and sterols which may be a reason for their antioxidant activity. The antioxidant property of medicinal plants has a different role in combating various diseases.

Keywords: Antioxidant, Gas chromatography-mass spectrometry analysis, Secondary metabolites

INTRODUCTION

Tridax procumbens is a widespread weed and pest plant which belongs to the family *Asteraceae* and is native to America. The plant is mostly scattered in tropical Africa, Asia, and Australia and is also found all over India.[1] It is usually named as "coat button." Various pharmacological studies on *T. procumbens* revealed that the whole plant has the properties to cure a variety of ailments, such as hair loss, bronchial catarrh, and dysentery and also prevents bleed from cuts.[2,3] Formerly it is showed that *T. procumbens* include hepatoprotective activity against anti-tuberculosis drug-induced hepatotoxicity in male Wistar rats,[4] along with that it also showed anti-inflammatory, immunomodulatory, hypoglycemic and anti-hyperglycemic effects,[5] wound healing, anti-hypertensive, antimicrobial, antiseptic, and anti-bradycardiac properties.[1,6] In addition to leaves, flowers are also a rich source of antioxidants.[7,8] Moreover, various metabolites have been reported earlier from essential oils of flower[9] and methanolic extracts.[10] Metabolite screening of a genus offers a systematic and in-depth understanding of phenotypic expression which in order, contributes to functional genomics, plant metabolite production, and physiology in addition to assimilating plant science among sustenance and

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human health.[11] Metabolomics is an expedient means to help out comprehension of a such comeback.[12] Therefore, profiling delivers information about the overabundance and presence of chemical constituents and thus is an efficient tool to find out such plants for novel bioactive molecules from plant resources.[13,14] It is very necessary to interpret the entire array of secondary metabolites of medicinal plants thus as to discover bioactive chemical compounds. Incomplete information on a plant's chemical composition restricts its applications in therapeutics role.

Seeing that, naturally occurring bioactive compounds such as secondary metabolites and their antioxidant properties are the potential sources of plants from traditional herbs, able to scavenge free superoxide radicals and can be useful in reducing the risk of various diseases.

Although all-embracing information occurs on the chemical composition of *T. procumbens*, this is a very limited study on hexane extracts of flowers of *T. procumbens*. However, the chemical, inherited, or ecological conditions result in a major transform in the chemical composition of a plant, therefore, the study of metabolites and antioxidant activity of *T. procumbens* could also support a complete and complete profiling of the medicinal herb. Phytochemicals such as α- limonene, β-sitosterol, and oleanolic acid are already present in other plants. However, the intention behind exploring its metabolites is that this plant will also be counted as one more resource of the same phytochemicals in the ethnobotanical list and contribute more to the bioavailability of these medicinal phytochemicals and to protect the plant species from being endangered which is caused by their over-exploitation for the therapeutic role.

Therefore, this study aimed to drive the research attention toward the flower parts of *T. procumbens* along with the leaves and stem parts. Hence, there will be full utilization of the medicinal properties of phytochemicals present in this medicinal herb.

The objective of the current study was to estimate *in vitro* antioxidant activity and metabolic profile of the medicinal herb *T. procumbens* leaves, stems, and flowers. Moreover, *T. procumbens* has not been reported to correlate with secondary metabolites using these extracts and solvent combinations.

MATERIALS AND METHODS

Plant Material

The plants were collected in the months of April-May from the fields of CSIR-Central Institute of Medicinal and Aromatic Plants Lucknow, India. The aerial part of the plant, that is, leaves, stem, and flowers was removed and washed with tap water and distilled water after that dried out at room temperature in shade for 7 days. The verification of plants was done and earlier the voucher specimen IU/PHAR/HRB/15/23 had been submitted at the herbarium of Pharmacognosy and Phytochemistry Department of Pharmacy, Integral University, Lucknow.

Extraction of Plant Material

The coarse powder of leaves (20 g) stem (20 g) and flowers (20) was subjected to successive extraction using hexane (non-polar) and methanol at room temperature overnight and filter out process repeated until the hexane layer became colorless after that the plant residue was air dried and kept into methanol for further extraction process repeated thrice. The combined filtrate obtained from hexane and methanol was concentrated in a vacuum evaporator (Buchi-200V, Switzerland) and stored at 4°C till further analysis.

DPPH Free-Radical Scavenging Assay

Hexane flowers, leaves, and stem; methanol flowers, leaves, and stem (hexane extract of *T. procumbens* flower [HTPF], MTPF, HTPL, MTPL, HTPS, and MTPS) extracts of *T. procumbens* were dissolved in methanol and different concentrations of sample solution (2, 10, 25, and 50 µg/mL) were made for the free radical scavenging activity of extracts. For free-radical scavenging assay, 50 µL of the sample solution was mixed with 100 mM Tris-HCl buffer (pH 7.4) and 500 µM of DPPH, incubated for 30 min in dark at room temperature and a decline in absorbance was recorded at 517 nm spectrophotometrically (SHIMADZU UV1240), a blank was prepared without adding extract and as compared to control percentage scavenging potential was analyzed by following the process described earlier.[15] Ascorbic acid was used as a positive control as the reference standard.

Reducing Power Assay

Reducing power assay is a very essential technique for the approximation of ferric reducing power of *T. procumbens* extracts. Different concentrations of extracts (2, 10, 25, and 50 µg/mL) were mixed with 200 mM phosphate buffer (pH 6.6) and 1% potassium ferric cyanide. The mixtures were incubated for 20 min at 50°C in a water bath and precipitated through 10% TCA (250 µL). The mixtures were centrifuged at 5000 rpm for 5 min and distilled water was mixed to an equal volume of the supernatant, the extracts with ferric reducing capacity were ensured by mixing 0.10% ferric chloride. The absorbance was recorded at 700 nm spectrophotometrically. Ascorbic acid was used as a standard. The increase in the reducing abilities of the reaction mixture is confirmed by an increase in the absorbance.

Nitric Oxide Scavenge Activity

The extracts of the *T. procumbens* at different concentrations (2, 10, 25, and 50 µg/mL) were mixed with sodium nitroprusside (10 mM in phosphate buffer saline) and

incubated for 30 min. at room temperature. The optical density was traced at 546 nm using a spectrophotometer[16] after adding 50 µL of the reaction blend to 100 µL of Griess reagent. The scavenging power of the extract was deliberated against a reagent blank taken as a control in terms of percent change.

Total Phenolic Content Evaluation

Total phenolic contents of *T. procumbens* extracts at different concentrations (2, 10, 25, and 50 µg/mL) were estimated by Folin–Ciocalteu reagent in terms of gallic acid equivalence.[17,18] Different concentrations of extracts were blended with Folin's reagent in dilution of 1:9 and $NaHCO_3$ (7.5%) pursued by incubation for 90 min at 37°C. The absorbance was recorded at 765 nm and the dark violet color showed the high phenolic substance of the extract. The phenolic substance was articulated as a gallic acid equivalent (GAE).[17] The data were represented as the mean of ±SD in triplicates.

Total Antioxidant Capacity (TAC) Assessment

The TAC of *T. procumbens* extracts was assessed as discussed in the procedure based on the reduction of Mo (VI) to Mo (V) in an acidic medium, as a result, a green color complex of phosphate and Mo^{+5} developed.[19] 1 mL TAC reagent prepared by 25 mM sodium phosphate monobasic, 635 mM ammonium molybdate, and 607 mM H_2SO_4, were mixed with different concentrations (2, 10, 25, and 50 µg/mL) of extract and heated for 90 min over hot water bath at 95°C. The produced optical density of the complex was considered by the use of a UV-Vis spectrophotometer at 695 nm against a reagent blank. The data were taken using ± SD. The TAC was expressed as ascorbic acid equivalent (AAE).[20]

Gas Chromatography-mass Spectrometry (GC-MS) Analysis

Identification of all non-polar (hexane) soluble and polar soluble (methanol) extract of leaves, stem, and flower was carried out by making TMS derivative to make them volatile. In GC grade C_5H_5N (20 mg mL^{-1}), 5–6 mg of the sample was mixed in 50 µL of CH_5NO. HCl (methoxyamine hydrochloride) solution. At 45°C, the blend was stirred for 2 h after that 70 µL of MSTFA was mixed and further shaken for 30 min. The lipid content was analyzed by GC-MS and carried out with Thermo Trace GC Ultra coupled through Thermo fisher DSQ II mass spectrometers. Chromatographic conditions were described earlier.[21] The Replib, mass spectral library (WILLY and NIST) was exercised to explore the resulting GC-MS profile by corresponding the chromatogram with commercially existing standards.

Electro Spray Ionization Mass Spectra (ESI-MS) Spectrometry

To determine the mass of the compound, the 2–3 mg of extracts were dissolved in chloroform and directly injected into the ESI MS. The mass spectrum has been obtained and analyzed for the parent ion to determine the molecular mass of the compound. Additionally, ESI-MS were traced by micro mass Quattro II instrument and the data are given in m/z values.[22]

Statistical Analysis

The comparative quantified data of metabolites obtained from polar (methanol) and non-polar (hexane) extracts of *T. procumbens* leaves, stems, and flower and the statistical significance for the relative area peak of GC-MS identified metabolites determined by SPSS (11.5.0, USA) between flower, leaves, and stem extracts. The statistical significance was expressed by $P \leq 0.05$.

RESULTS

DPPH Free-Radical and NO Scavenging Activity

In the case of non-enzymatic components, activity for free-radical scavenge was performed by DPPH assay. The results indicated that DPPH free radicals were inhibited by flower hexane extract (HTPF), flower methanolic extract (MTPF), and leave methanolic extract (MTPL) with an IC_{50} 18.5 µg/mL, 33.14 µg/mL, and 34.52 µg/mL, respectively [Figure 1a].

However, the scavenging activity of nitric oxide of *T. procumbens* extracts is measured as a percent of NO scavenge in which a maximum of 67.32% nitric oxide were observed in HTPF, 58.79% in MTPF, 56.12% in MTPL, and 39.12% in HTPL, respectively, at maximum concentration 50 µg/mL. In comparison to methanolic extracts, HTPF has shown the most significant nitric oxide scavenging activity which was indicated by its IC_{50} value HTPF 16.74 µg/mL, MTPF 24.60 µg/mL while MTPL with an IC_{50} 34.26 µg/mL, respectively, whereas ascorbic acid taken as standard has an IC_{50} value 27.26 µg/mL [Figure 1b] The reduction potential of flower and leaves of *T. procumbens* ranges in the order of HTPF >MTPF >HTPL >MTPL> MTPS>HTPS [Figure 1c] in contrast with quercetin (0.92 ± 0.01) at 50 µg/mL.

The TAC of hexane extracts of flowers, leaves (HTPF, HTPL, and HTPS) and methanolic extracts of flowers, leaves (MTPF, MTPS, and MTPF) of *T. procumbens* were tested against AA in a dose-dependent manner (50 µg/mL). The HTPF showed a maximum TAC of 16.22AAE followed by HTPL 15.07AAE at a concentration of 50 µg/mL [Figure 1d].

The consequences of the current study explored the phenolic content of HTPF, HTPL, MTPL and MTPF by using

Activities	HTPF	MTPF	MTPL
DPPH Activity IC50(μg/mL)	18.5	33.14	34.52
NO Scavenging Activity IC50(μg/mL)	16.74	24.60	34.26
Total Antioxidant Activity (AAE)	16.22	15.07	14.02

Figure 1: Concentration-dependent (a) DPPH free radical inhibition, (b) nitric oxide inhibition, (c), reducing power assay (d) total antioxidant capacity and (e) total phenolic activity of hexane and methanolic extracts of *Tridax procumbens* flowers, leaves, and stem, values are mean ± SD of three individual tests in replicate at each concentration

Folin-Ciocalteu and expressed in terms of GAE. The total phenolic contents ranged from 8.32 ± 0.18 to 4.04 ± 0.11 GAE [MTPF> MTPL > HTPF > HTPL, Figure 1e].

Comparative metabolite profiling of leaves, stem and flower samples of *T. procumbens* was performed using GC-MS. Hundreds and more chemically different metabolites consisting of fatty acids, terpenes, phenolics, steroids, sterols, and α-tocopherol were characterized in hexane (non-aqueous) extracts from various parts of *T. procumbens*. Student *t*-test revealed the significant distinction in the concentration of oleanolic acid, β-Sitosterol, stigma sterol, 2, 4-di-*tert*-butyl phenol germacrene, N-acetyl glucosamine, and *trans* farnesol derivative between leaves stems and flowers of *T. procumbens*. Oleanolic acid was detected as one of the major metabolites in the non-aqueous extract of leaves, stems, and flowers. The percent peak area of oleanolic acid was found 32.13 ± 0.3.11% (leaves), 17.66 ± 1.71% (flowers), and 12.63 ± 1.22% (stems), respectively. Oleanolic acid [(3β)-3-hydroxyolean-12-en-28-oic acid] is a natural pentacyclic triterpenoid. Relatively high quantities of β-Sitosterol 5.86 ± 0.59% in leaves, 2.46 ± 0.25% and in flowers 1.45 ± 0.14% were detected in non-aqueous extract of the *T. procumbens*. It was detected another compound stigma sterol comparatively more in leaves 4.56 ± 0.41%, than at 3.22 ± 0.31% (stems) and 2.85 ± 0.23% (flowers), respectively.

Relatively, high amount of *trans*-farnesol was discovered from non-aqueous (hexane) extract of *T. procumbens* flowers than leaves and stems (4.56 ± 0.45%, 2.33 ± 0.21%, and 1.56 ± 0.11%).

Phytochemicals have been imperative since antiquity for curing various ailments and have also proven to be a significant antioxidant activity. Subsequently, in recent years, the identification and development of drugs based on natural products have emerged as a leading field in various diseases. The concentration of 2, 4-di-*tert*-butyl phenol in non-aqueous extracts of leaves was 2.98 ± 0.31%; in flowers 1.34 ± 0.12% and a very small amount in stems 0.56 ± 0.05% was found. However, the germacrene in leaves was detected higher in leaves (2.22 ± 0.22) almost twice in flowers (1.23 ± 0.14) and a very trace amount was present in stems (0.12 ± 0.01). The percent peak area of cholesterol in leaves, flowers and stems was 2.22 ± 0.22; 1.89 ± 0.19 and 1.56 ± 0.15, respectively. Relatively, the concentration of the two major fatty acids palmitic and linolenic acids were maximum in stems (9.52 ± 0.89 and 2.79 ± 0.31) which was nearly four times higher than that of flowers (1.85 ± 0.19 and 1.22 ± 0.12) and very little quantity was found in leaves (1.63 ± 0.16 and 2.12 ± 0.25). A significantly high percent peak area of stearic acid was found in stems 2.56 ± 0.31 leaves1.89 ± 0.21 and in flowers 1.28 ± 0.12. Moreover, its non-aqueous fraction of leaves, stem, and flowers showed terpenes such as δ-element, β-element, γ-element, and β-Cubebeneas as the important metabolites in traces. A very high concentration of fructose in the methanolic extract of stems was 36.5 ± 3.11; in flowers 28.96 ± 2.11 while in leaves it was measured at 22.56 ± 2.11. However, the glucose percent peak area was high in leaves 22.56 ± 2.11 in contrast with stems 15.4 ± 2.11 and flowers 13.56 ± 2.11. The percent peak area for malic acid was high in methanolic flower extract 12.44 ± 1.11; in leaves extract and 11.25 ± 1.13 in flowers, respectively. The fumaric acid and L-proline were high in extracts of leaves at 6.25 ± 0.61 and 4.25 ± 0.44. The sucrose concentration was high in leaves 3.21 ± 1.12; 2.33 ± 0.98 in stems and 1.45 ± 1.00 in flowers, respectively. The maltose concentration was high in flowers at 8.33 ± 1.12 than in leaves at 6.77 ± 1.45; stems at 6.12 ± 1 as well. A very small quantity of ribitol, xylulose, N-Acetyl glucosamine, cellobiose, and mannitol was also found in the leaves stems and flowers of *T. procumbens* [Tables 1-3].

DISCUSSION

Naturally, free radicals are formed in the body and contributed a significant role in numerous common cell processes[23] at elevated concentrations; conversely, free radicals can be harmful to the body and can injure all major cell components such as DNA, proteins and cell membranes. Free radicals caused cellular damage as well as destruct the DNA which creates lethal diseases like cancers.[23] Therefore, natural compounds with free radical scavenging activity have been found to play a protective role in maintaining normal healthy cells and can also be taken as an adjuvant to the therapy resulting in symptomatic relief.[24] The previous reports and our experimental findings have shown that HTPF was very effective in inhibiting DPPH free radicals in a dose-dependent mode comparable to that of ascorbic acid. Similar results were found in other studies where the presence of both enzymatic, as well as non-enzymatic components; confirm the potent antioxidant potential of plant extracts. Moreover, nitric oxide is a ubiquitous free radical signaling molecule generated by endothelial cells, macrophages, and neurons which helps to regulate various cellular processes together with apoptosis, immune response, and angiogenesis, etc.[25] Therefore, nitric oxide assay has been performed to measure the alteration of structural and functional activities of several cellular components. Since, nitric oxide or ROS produced in

Table 1: Qualitative and quantitative variation in non-aqueous (hexane) metabolites among three different parts of Tridax procumbens using GC-MS

tR (min)	Metabolite	Leaves	Stem	Flower
8.06	α-Limonene	0.55±0.05$^{a\ b}$	ND	ND
9.26	Propanoic acid	0.48±0.05^{a,b}	5.77±0.50^{c}	11.9±1.12
9.83	Glycerol	1.21±0.11^{a,b}	2.90±0.31^{c}	3.56±0.35
10.58	Propylene Glycol	1.45±0.14$^{a\ b}$	2.03±0.21^{NS}	2.79±0.31
14.29	δ-Elemene	0.2±0.02^{a,b}	0.11±0.01^{c}	ND
15.62	Nonanoic acid	0.69±0.07^{a,b}	2.28±0.21^{c}	0.23±0.02
15.64	γ-Elemene	1.10±0.11^{Ns}	0.78±0.07^{c}	1.20±0.14
18.7	α-Caryophyllene	0.67±0.07^{b}	0.23±0.02^{c}	ND
18.84	β-Cubebene	0.34±0.03^{a}	0.12±0.01^{c}	0.21±0.02
20.39	2,4-di-tert-butyl phenol	2.98±0.31^{a,b}	0.56±0.05^{c}	1.34±0.12
20.63	Hexadecene	0.71±0.07^{a,b}	0.05±0.00^{c}	0.12±0.01
22.67	Lauric acid	0.43±0.04^{b}	0.31±0.03^{c}	0.05±0.00
24.75	Undecanedioic acid	1.98±0.21^{a,b}	0.22±0.02^{NS}	0.31±0.03
25.1	Eicosene	1.18±0.11^{a,b}	2.9±0.30^{c}	0.11±0.01
25.5	cis-5,8,11-Eicosatrienoic acid	0.26±0.02^{a,b}	ND	0.54±0.05^{c}
26.81	Myristic acid	1.35±0.13$^{a\ b}$	0.23±0.02^{c}	0.44±0.04
26.83	Germacrone	2.22±0.22^{a,b}	0.12±0.01^{c}	1.23±0.14
28.03	Hexadecanol	0.34±0.03^{a,b}	2.03±0.21^{c}	ND
28.7	Dodecylsuccinic acid anhydride	1.56±0.15^{a,b}	2.28±0.25^{c}	0.11±0.01
29.13	Docosene	1.73±0.17^{a,b}	0.02±0.00^{c}	0.25±0.02
30.62	Palmitic acid	1.63±0.16^{a}	9.52±0.89^{c}	1.85±0.19
32.15	trans-Farnesol deriv.	2.33±0.21^{a,b}	1.56±0.11^{c}	4.56±0.45
33.66	Oleic acid	1.98±0.22^{a,b}	0.23±0.02^{c}	0.31±0.03
34.11	Stearic acid	1.89±0.21^{a}	2.56±0.31^{c}	1.28±0.12
34.35	Linoleic acid	2.12±0.25^{NS}	2.79±0.31^{c}	1.22±0.12
34.82	α-Linolenic acid	1.12±0.11^{a,b}	2.23±0.19^{c}	0.41±0.11
36.25	Hexacosene	0.54±0.05^{a,b}	0.03±0.00^{c}	0.08±0.02
39.4	Pentatriacontene	0.28±0.03^{b}	0.11±0.10^{c}	0.02±0.00
41.95	Corticosterone	0.57±0.05^{NS}	0.69±0.07^{NS}	0.64±0.06
42.32	Oleanolic acid	32.13±3.11^{a,b}	12.63±1.22^{c}	17.66±1.71
42.93	Ursa-9 (11),12-dien-3-one	2.78±0.21^{a,b}	0.55±0.05^{c}	0.23±0.23
47.21	Cholesterol	2.22±0.22^{a,b}	1.56±0.15^{NS}	1.89±0.19
48.11	Triacontanol	0.80±0.08^{a,b}	ND	0.12±0.01^{c}
48.13	α-Tocopherol	0.23±0.02^{a,b}	ND	ND
49.51	Campesterol	0.66±0.06^{a}	0.14±0.01^{c}	0.89±0.09
49.82	Stigmasterol	4.56±0.41^{b}	3.22±0.31^{c}	2.85±0.23
50.68	β-Sitosterol	5.86±0.59^{a,b}	2.46±0.25^{c}	1.45±0.14
51.45	β-Amyrin	2.66±0.26^{a,b}	1.23±0.12^{NS}	1.48±0.14

Mean value±SD of relative percentage peak area; NS, a, b, c statistical significance $P \leq 0.05$ within leaves stem and flower where a=leaves versus stem, b=leaves versus flower, c=stems versus flower NS $P>0.05$. GC-MS: Gas chromatography-mass spectrometry

their reaction through oxygen or superoxides, as very reactive NO_2, N_2O_4, and N_3O_4. The results of these analyses show that this plant is an enriched source of natural antioxidants which supports the previous reports.[26]

Often, naturally occurring compounds belonging to poly phenol sub-family of natural products contribute to combatting various diseases such as cardiovascular diseases, type 2 diabetes, pancreatitis, gastrointestinal

Table 2: Qualitative and quantitative variation in aqueous (methanol) metabolites among three different parts of Tridax procumbens using GC-MS

Rt (minute)	Metabolite	Leaves	Stem	Flower
11.24	Glycerol	1.22±0.12[a]	2.36±0.21[c]	1.02±0.11
14.29	L-Proline	4.25±0.44[b]	3.21±0.33[c]	1.56±0.12
15.5	Fumaric acid	6.25±0.61[a]	3.22±0.29[c]	5.42±0.54
18.72	Malic acid	11.25±1.13[a]	8.93±0.85[c]	12.44±1.11
18.84	Xylulose	1.22±0.13[a,b]	0.23±0.02[c]	0.45±0.04
21.24	Xylitol	0.52±0.05[b]	0.88±0.08[c]	0.21±0.02
23.66	Ribitol	1.12±0.11[b]	1.86±0.14[c]	2.54±0.25
24.12	Mannitol	0.22±0.02	0.54±0.05	0.36±0.04
24.87	Fructose (1 TMS)	22.56±2.11[a,b]	36.5±3.11[c]	28.96±2.11
25.11	Fructose (2 TMS)	4.56±1.12[a,b]	5.22±1.23[a]	4.85±1.22
25.33	Glucose (1 TMS)	18.74±2.14[a,b]	15.4±2.11[b]	13.56±2.11
25.68	Glucose (2 TMS)	6.52±1.13[a]	4.66±1.09[c]	7.12±1.14
27.65	Galactose	1.45±0.89[b]	2.56±0.98[c]	1.12±1.00
28.61	N-Acetyl glucosamine	0.65±0.04[a,c]	0.32±0.01[a]	0.84±0.07
31.02	Gluconic acid	0.23±0.02[b,c]	1.24±0.40[c]	1.11±0.34
42.34	Maltose	6.77±1.45[a,b]	8.33±1.12[a]	6.12±1.11
46.25	Sucrose	3.21±1.12[a,b]	2.33±0.98[a]	1.45±1.00
51.22	Cellobiose	0.65±0.45[a,c]	0.35±0.2[c]	1.02±0.67

Mean value±SD of relative percentage peak area; NS [a, b, c] statistical significance $P \leq 0.05$ within leaves stem and flower where a=leaves versus stem, b=leaves versus flower, c=stems versus flower NS $P>0.05$. GC-MS: Gas chromatography-mass spectrometry

Table 3: Mass fragmentation of GC-MS identified metabolites from Tridax procumbens

Compound name	tR (min)	Molecular	Mass fragmentation
α-Limonene	8.06	$C_6H_{14}O_2Si$	m/z 146(M^+), 131, 117, 99, 75, 73
Propanoic acid		$C_{12}H_{32}O_3Si_3$	m/z 308 (M^+), ,218, 206,205, 147, 133 129, 117, 103, 73,59,45 53
Glycerol	9.83	$C1_2H_{32}O_3Si_3$	m/z 308(M^+), 293(M^+-CH3), 205, 1 47, 133, 103, 101, 73 (100%)
Propylene Glycol	10.58	$C_9H_{24}Si_2$	m/z 220(M^+), 147, 117, 73
δ-Elemene	14.29	$C_{10}H_{16}$	m/z 136(M^+), 121, 93, 79 (100%), 68, 52
Nonanoic acid	15.62	$C_{12}H_{26}O_2Si$	m/z 230(M^+), 215(M^+-CH3) (100%), 171, 145, , 117, 73 ,55
γ-Elemene	15.64	$C_{15}H_{24}$	m/z 204(M^+), 189, 161, 147, 121, 93, 81 (100%), 68, 55, 41
α-Caryophyllene	18.7	$C_{15}H_{24}$	m/z 204(M^+), 189, 161 (100%), 133, 120, 107, 93, 79, 69
β-Cubebene	18.84	$C_{12}H_{32}O_3Si_3$	m/z 308(M^+), 293(M^+-CH_3), 205, 147, 133, 103, 101, 73 (100%)
2,4-di-tert-butyl phenol	20.39	$C_{17}H_{30}OSi$	m/z 278(M^+), 263(M^+-CH3) (100%), 73, 57
Hexadecene	20.63	$C_{16}H_{34}$	m/z 226(M^+), 183, 127, 113, 85, 71, 57 (100%),
Lauricacid	22.67	$C_{15}H_{32}O_2Si$	m/z 272(M^+), 257(M^+-CH3), 145, 132, 117 (100%), 73, 55
Eicosene	25.1	$C_{20}H_{40}$	m/z 280(M^+), 252, 182, 111, 97 (100%), 83, 69, 43
Myristic acid	26.81	$C_{17}H_{36}O_2Si$	m/z 300(M^+),285(M^+-CH3), 201, 145, 132, 117, 73 (100%),43
Germacrone	26.83		m/z 218(M^+), 203, 175, 135,121, 107, 91, 82, 67, 53
Hexadecanol	28.03	$C_{19}H_{42}OSi$	m/z 314(M^+), 209, 103 (100%), 75, 43
Dodecylsuccinic acid anhydride	28.7	$C_{21}H_{44}O_2Si$	m/z 356(M^+), 221(M^+-Me),206, 179, 149, 103, 73
Docosene	29.13	$C_{22}H_{44}$	m/z 308(M^+), 145, 132, 117, 111, 73, 55 (100%)
Palmitic acid	30.62		m/z 328(M^+), 313(M^+CH3), 285, 269, 243, 117 (100%), 73
trans-Farnesol deriv.	32.15	$C_{18}H_{34}OSi$	m/z 294(M^+), 279(M^+-CH3), 257, 229, 143, 107, , 69 (100%)

(*Contd*....)

Table 3: *(Continued)*

Compound name	tR (min)	Molecular	Mass fragmentation
Oleic acid	33.66	$C_{21}H_{42}O_2Si$	m/z 354(M^+), 339(M^+-CH3), 199, 145, 129, 117, 73 (100%)
Stearic acid	34.11	$C_{21}H_{44}O_2Si$	m/z 356(M^+), 341(M^+-CH3), 328, 297, 147, 145, 117 (100%), 97, 73
Linoleic acid	34.35	$C_{21}H_{40}O_2Si$	m/z 352(M^+), 337(M^+-CH3), 262, 129, 95, 73 (100%)
α-Linolenic acid	34.82	$C_{21}H_{38}O_2Si$	m/z 350(M^+), 335(M^+-CH_3), 280, 149, 129, 95, 73
Hexacosene	36.25	$C_{29}H_{68}$	m/z 416 (M^+), 317(M^+-CH_3), 292, 73
Pentacosanoic acid	39.4	$C_{28}H_{58}O_2Si$	m/z 454(M^+),439(M^+-CH3), 395, 201, 145, 132, 117 (100%), 97, 73
Corticosterone	41.95	$C_{27}H_{46}O_4Si_2$	m/z 490(M^+), 307, 269, 143, 103, 73
Oleanolic acid	42.32	$C_{30}H_{48}O_3Si$	m/z 457(M^+, 439, 411, 393, 248 (100), 217, 203, 177, 163
Cholesterol	47.21	$C_{30}H_{54}OSi$	m/z 458(M^+), 443(M^+-CH3), 368, 329, 247, 129 (100%), 73
α-Tocopherol	48.11	$C_{32}H_{58}O_2Si$	m/z 502(M^+), 486, 388, 397, 277, 237 (100%), 73
Triacontanol		$C_{33}H_{70}OSi$	m/z 510(M^+), 509, 495(M^+-CH3) (100%), 409, , 222, 103, 97, 75, 57
Campesterol		$C_{31}H_{56}OSi$	m/z 472(M^+),457(M^+-CH3), 382, 255, 129 (100%), 73
Stigmasterol	49.82	$C_{32}H_{56}OSi$	m/z 484(M^+),469(M^+CH3), 394, 255, 217, 147, 129, 83 (100%),73
β-Sitosterol	50.68	$C_{32}H_{58}OSi$	m/z 486(M^+), 471(M^+-CH3), 396, 280, 217, 147, 73 (100%)
β-Amyrin	51.45	$C_{33}H_{58}OSi$	m/z 498(M^+), 218(M^+-CH_3), 203, 190, 95, 73
L-Proline	14.29	$C_{11}H_{25}NO_2S_2$	m/z 259(M^+), 216, 147,142, 121, 86, 73
Malicacid	18.72	$C_{13}H_{30}O_5Si_3$	m/z 350(M^+), 255(M^+-CH_3), 245, 233, 147, 73
Xylulose	18.84	$C_{18}H_{45}NO_2S_4$	m/z 457, 354, 263, 206, 173, 147,133, ,103
Xylitol	21.24	$C_{20}H_{52}O_5Si_5$	m/z 512(M^+), 319(M^+-CH_3), 307, 217, 205, 147, 129, 103, 73
Ribitol	23.66	$C_{20}H_{52}O_5Si_5$	m/z 512(M^+), 319, 307, 217, 205, 147, 129, 117, 103, 73
Mannitol	24.12	$C_{24}H_{62}O_6Si_6$	m/z 614(M^+), 319(M^+-CH_3), 217, 205, 147, 117, 103, 73
Fructose (1 TMS)	24.87	$C_{21}H_{52}O_6Si_6$	m/z 540(M^+) 437, 217, 204, 147 (100), 129, 73
Fructose (2 TMS)	25.11	$C_{21}H_{52}O_6Si_5$	m/z 540(M^+, 100%), 433, 217, 204, 147, 129
Glucose (1 TMS)	25.33	$C_{24}H_{61}NO_6Si_6$	m/z 627(M^+), 319(M^+-CH_3), 229, 218, 205, 147, 117, 103, 73
Glucose (2 TMS)	25.68	$C_{24}H_{61}NO_6Si_6$	m/z 627(M^+), 319(M^+-CH_3), 229, 205, 147, 117, 103, 73
Galactose	27.65	$C_{24}H_{61}NO_6Si_6$	m/z 627(M^+), 319, 219, 205, 147, 103, 73
N-Acetyl glucosamine	28.61	$C_{21}H_{50}N_2O_6Si_4$	m/z 538, 319, 206, 147, 129, 103, 73
Gluconic acid	31.02	$C_{18}H_{42}O_6Si_4$	m/z 466(M^+), 217, 147, 103, 73
Maltose	42.34	$C_{36}H_{66}O_{11}Si_8$	m/z 918, 361, 217, 204, 191, 147, 103,73
Sucrose	46.25	$C_{36}H_{86}O_{11}Si_8$	m/z 918, 432, 361, 319, 217, 147, 103,73

M^+: Molecular ion peak. GC-MS: Gas chromatography-mass spectrometry

illness neurodegenerative problems, certain cancers, and lung infection.[27-30] The polyphenolic compounds control the formation of free radicals with the production of stable complexes. Polyphenols produced hydrogen peroxide that helps to protect from oxidative damage.[31,32]

However, from GC-MS the metabolite profiling of leaves, stem and flower samples of *T. procumbens* showed the presence of hundreds and more chemically different metabolites such as fatty acids, terpenes, phenolics, steroids, sterols, and α-tocopherol. In vegetables and medicinal plants, oleanolic acid was supposed to be the most significant compound for bioactivity.[33] It has been previously described for its anti-tumor activity.[27] The previous reports also suggested that OA has anti-inflammatory, antiviral, and hepatoprotective efficacy.[34] Another study showed that OA has no cytotoxicity against normal cells.[33] On a similar note, β-sitosterol has been distinguished for the anti-tumor activity of the liver, lung cancer, colon, stomach, breast, and prostate cancers.[35] It exhibited cytotoxicity through cell cycle arrest and apoptosis induction.[36]

Previously reported document showed that *trans*-farnesol possess chemopreventive properties through modulating tumor cell growth.[37] They have a significant role in reducing the threat of malignancy.[38,39] In *Tridax* species, several metabolites such as 2, 4-di-*tert*-butyl phenol, germacrene, N-acetyl glucosamine, and *trans* farnesol derivative were present which have not been reported earlier. Thus, this study is addressing these metabolites for the first time in *T. procumbens*.

The alkenes such as dodecane, tetradecane, hexadecene, octadecene, and alkylated phenols, isochrone derivatives and sterols have been seen in nonaqueous extracts of leaves stems and flowers. Various sugar moieties have been found such as glucose, fructose, galactose, and many more. The *t*-test analysis showed significant differences in concentrations of polar metabolites in the different plant ($P < 0.05$) parts such as leaves stem and flowers.

CONCLUSION

Due to these characteristics, there is a need for further research to explore its activity. To fulfill this need our experimental findings regarding the antioxidant activity of HTPF suggest that this vital property is due to the presence of aforesaid secondary metabolites content identified by GC-MS analysis. Thus, the antioxidant activity of HTPF provides an initial lead that the metabolites involved in hexane fraction would be an effective therapeutic agent against various diseases.

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