Current Trends in Biotechnology and Pharmacy
Vol. 15 (5) 444-451, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2021.3s.38

Anti-inflammatory attributes using Protein denaturation by Decanoic acid (Saturated fatty acid) Isolated and Identified from *Tridax-procumbens L*

Hanumantappa Bherigi Nayaka*, Lallungawii K, Malsawmtluangi, Mary M.S.

Department of Life Sciences, Kristu Jayanti College Autonomous, Bengalore-77, Karnataka, India

***Corresponding author:** hbnayaka3@gmail.com

Abstract

Tridax procumbens is a common herb with significant medicinal properties traditionally used in the treatment of many diseases. In the present investigation the compound isolated from ethanol extract of *T. procumbens* exhibited high antifungal, against clinically important human pathogens such as *Candida albicans* and *Malassezia sp* with low MIC values, the compound later evaluated for anti-inflammatory activity by protein denaturation method and showed moderate results, than the compound also tested for clot Lysis activity and the results indicated that the compound has a good clot Lysis activity. The fractionation of ethanol extract with dichloromethane yielded an oily viscous semi-solid drug with Antifungal, Anti-inflammatory and Clot Lysis activities which was separated by PTLC and Column chromatography and subjected to further identification and structural elucidation by spectral analysis such as FT-IR, Proton NMR and GC–MS, Spectral analysis and interpretation of spectral data revealed that the isolated compound was the Decanoic acid (Saturated fatty acid. This study demonstrated the efficacy of this herb and isolated compound against clinically important Dermatophytes, Anti-inflammatory and Clot Lysis activity. These research findings also justified that the *T. procumbens* was strong traditional medicinal plant used by our ancestors in India.

Keywords Anti-inflammatory, *Tridax procumbens L,* Decanoic acid, Clot Lysis

Introduction

Tridax procumbens is a weed that is flowering plants widely spread and commonly referred to as “Tridax daisy” or “coat buttons”. It is a species of the Asteraceae family and is native to the Central and South America (1, 2) and is widely spread all over the tropical and sub-tropical area of Africa, Asia, etc. They are usually seen growing with crops, near ponds, along roadsides, pastures land, and waste areas. *T. procumbens* has been used in Ayurveda since the Ancient time in India (3). In India, *T. procumbens* is common and locally known as “Ghamara” or “Jayanthi” in Ayurveda/Sanskrit. In India It is used as wound healing, anti-coagulant, anti-fungal, insect repellent, anti-hair fall and for treating diarrhea. Study also shows that it is used as remedy for liver disorders and antidiabetic activity. *T. procumbens* has demonstrated significant anti-inflammatory and antimicrobial activity (4-11). *T. procumbens* is straggling herb that measure about 12 - 24cm long with few leaves 6-8cm long and very long slender solitary peduncles a foot long and more. Leaf is simple, opposite, estipulate, ovate, acute, inflorescence capitulum. *Tridax* has two types of flowers ray-florets and disk-florets, Basal placentation, fruit is cypsela. The plants has a daisy like yellow which is located to the center of the flower and have a toothed ray florets which is white in color. The leaves are toothed and generally have an arrowhead- shape. *T. procumbens* is classified as a poisonous weed in Alabama, Florida, Minnesota, North and South Carolina and Vermont. It is quarantined in California and Oregon and prohibited in Massachusetts (U.S. Department of Agriculture).

Due to the plant’s defense mechanisms and their secondary metabolites such as flavonoids, alkaloids, tannins, carotenoids, saponins, oils and fatty’s this species seem to have a versatile character and can be used as a drug to eliminate many diseases. Screening of phytochemical test revealed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones) and tannins also carotenoids and saponins. *Tridax* leafs mostly contains 26% crude proteins, 17% crude fiber, 39% soluble carbohydrates, 5% calcium oxide. Its flower has been reported to have Luteolin, glucoluteolin, quercetin and isoquercetin. While the plant is reported to be have fumaric acid, fl-sitosterol and tannin. Oleanolic acid was obtained in good amounts from *Tridax* and they are found to have potential antidiabetic activity when tested against aglucosidase (12). They have a multiple chemical constituents like alkaloids, carotenoids, tannins, flavanoids like quercetin, saponin. The mineral that are present in the leaves are calcium, magnesium, potassium, sodium and selenium. The secondary metabolites of *T. procumbens like* flavonoids, terpenoids and alkaloids are used as drugs or to avert a variety of diseases at present. Researchers pay more attention to Fatty acids for research and development consideration, since the compounds have the able to protect the plants from stress and oxidative reaction and also provide protection to human being from free radicals cascade due to their pharmacological properties like antioxidants, hepatoprotective, antimicrobial, antiallergic, analgesic and anti- inflammatory, anticarcinogenic and anti- obesity activities. Current scenario requires more attention for safety, effectiveness and quality of medicinal products

from plants and herbs. Thus, it becomes necessity to identify and quantify all the secondary metabolites to ensure the pharmacological research repeatability and reliability along with maintaining the quality control on pharmacological merits or demerits.

With all this above information, in the present study focused on the isolation and identification saturated fatty acid (Decanoic acid) and also pharmacological evaluation of isolated compound such as antifungal activity, anti-inflammatory activity, and clot Lysis activity to develop possible drug for the respective diseases and disorders which are related to these activities.

Materials and Method

Collection of Plant Material

The leaves of *T. procumbens* were collected from in and around of Kristu Jayanti College, Kothanur Bangalore during the month of October 2020. The leaves were separated and washed under running tap water to remove dust particle and then shade dried at room temperature for about three weeks and the dried leaves were made into powder using pestle and motor for further extraction of crude drug.

Extraction of Crude drug

During extraction, 100 g of powered sample was used for extraction with different from non-polar to polar solvents such has Petroleum ether, Chloroform and Ethanol with their respective boiling point until solvents become color less for 5-6 hours. The crude extracts so obtained in 3 different solvents were evaporated in three separate watch glasses or desiccator to remove the solvent, once the evaporation completed semi solid crude drug obtained has collected and stored in refrigerator for further use.

Qualitative analysis of Phytochemicals

Preliminary phytochemical screening and quantitative test for the presence of phenols, tannins, flavonoids, alkaloids and fatty acids was carried out using standard test protocols. These phytochemicals were identified by characteristics color change using standard procedures (13).

Qualitative Test for Alkaloids:

Wagner's Test is the most common test used for qualitative test for alkaloid and in this test the few drops of Wagner's reagent were added into 2 to 3 ml of extract, the Formation of reddish brown precipitate indicated the presence of alkaloids (14).

Qualitative test for Oils and Fats

Oils and fats are long chain esters of fatty acids, oils are unsaturated esters of fatty acids and fat are saturated fatty acids. E.g.: Steric acid, laconic acid etc. the spot test and saponification was the test used for qualitative test for oils and fats. In spot test a small quantity of extract is pressed with filter paper if an oils stain on the paper indicates the presence of oils or fats but where in case of saponification test to a small quantity of extract few drops of 0.1N KOH and phenolphthalein and heated on water both for 30 min. the formation of soaps after 30 min heating indicates the presence of oils or fats.

Qualitative Test for Phenols

Phenols are the aromatic compounds in which OH (hydroxyl) group is directly attached to benzene ring depending on the attachment of OH Group to benzene ring the phenols are classified. E.g.: Cresols, Catechol, etc., Ellagic acid test is most comely used for phenolic test in this test to the extract 3 drops of 5% NaNH2 solution is added and observe the formation of Muddy/ Niger brown precipitate (15).

Qualitative Test for Flavonoids

Two different methods were used to determine the presence of flavonoids. The Shinoda test is most commonly used for flavonoids and in this test, to the extract; 5 mL of dilute ammonia solution was added to a portion of total flavonoid extracts followed by addition of concentrated H_2SO_4. The yellow color is formed after adding H_2SO_4 and is disappeared on standing. Few drops of aluminum solution added and again gained the yellow color (16, 17).

Qualitative Test for Tannins

The tannin compounds are widely distributed in many species of medicinal plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation. Ferric Chloride test is most commonly used for tannins in this test, to the Extract added 1% Ferric chloride solution and observed the formation of the Blue green or brownish green color (18).

Separation of compounds using PTLC:

Preparative Thin Layer Chromatography (PTLC) was done for the preliminary separation of compounds. The Stationary Phase used is the Silica Gel pre-coated in TLC and with Toluene: Chloroform: Methanol (4:4:2) were used as Mobile Phase After the plate developed, it was air dried at room temperature, then placed in Iodine Chamber to detect and visualization of compounds separated. The spot color was observed and calculated the Rf value using the following formula (19).

$$\text{RF value} = \frac{\text{Distanced travelled by the solute}}{\text{Distanced travelled by the solute}}$$

Separation of compounds by Column Chromatography

The compound which was isolated can be purified and separated by column chromatography separation

method. The 45 cm length and 3 cm width of the column was used and it is filled with the slurry of silica gel-H of mesh size 60–120 μ (Hi media, Mumbai) to 1/3 portion using n-hexane. Care should be taken to avoid the air bubble formation during column packing. Set the column by the solvent n-hexane. 1 g of isolated compound was bound with silica gel and loaded on the top of the column. The column was eluted with gradient solvent system of Toluene: Chloroform: Methanol (4:4:2) as Mobile Phase v/v/v until the color of the column is colorless.

Pharmacological Activities

Antifungal activity by cup plate method

In antifungal activity the cup plate Method is used with standard fungal cultures of Candida and Malassezia sp. The different concentrations of compound such as 25ug/ml, 50ug/ml, 100ug/ml was prepared and Amphotericin B antibiotic used as positive control in this assay. Potato Dextrose Agar plates with these fungal cultures were made the 4 different wells in which one well used as positive control by loading Amphotericin B and remaining 3 wells used for different concentration of isolated compound, than incubate the at room temperature for 2-3 days (20).

Anti-inflammatory activity by Protein denaturation method

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from hen's egg), 2.8 ml of Phosphate Buffered Saline (PBS, pH6.4) and 2ml of varying concentrations of isolated compound from Ethanol extract *of T. procumbens* L., so that final concentration become 31.25, 62.5, 125, 250, 500, 1000 µg/ml, similar volume of double distilled water served as a control. Then the mixtures were incubated at 37±2 °C for 15 minutes and then heated at 70 °C for 5 minutes. After cooling, their absorbance was measured at 660 nm, by using vehicle as blank. Diclofenac sodium at final concentration of (31.25, 62.5, 125, 250, 500, 1000 µg/ml) was used as reference drug for positive control. The inhibition percentage of protein denaturation was calculated by using following formulae (21).

% of inhibition = 100 x (Vt/Vc– 1)

Where, Vt = absorbance of test sample

Vc = absorbance of control

The compound/ drug concentration for 50 % inhibition (IC50) was determined by plotting percentage inhibition with respect to control against concentration.

Clot Lysis activity by euglobulin clot Lysis method

In clot Lysis activity, venous bloods is collected and distributes in 5 different pre-weight sterile centrifuge tubes (0.5ml/tube) and incubate for 45 minutes at 37˚C. After the formation of clot the serum is removed from the tube without disturbing the clot. The tubes with the blood clot is weight to determine the clot weight (clot weight = weight of tube with blood clot – weight of empty tube). Add 100ul of the plant extract having different concentration of 25ug/ml, 50ug/ml and 100ug/ml in each tube and use 100ul of distilled water as negative control and add 2.5ml of PBS to lyophilized streptokinase and use as positive control. Incubate at 37˚C for 90 minutes the remove the fluids form the tube and weight to observe the weight difference after clot distribution. Difference obtained in weight taken before and after were expressed as percentage (%) of clot Lysis (22).

Identification and Structural Elucidation of isolated compound by Spectral Analysis

FT-IR: Fourier Transform Infrared Spectroscopy (FT-IR) is an analytical technique used to identify organic, polymeric, and, in some cases, inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties.

In FTIR analyses, Infrared light from the light source passes through a Michelson interferometer along the optical path. The Michelson interferometer comprises a beam splitter, moving mirror, and fixed mirror.

Fourier transform infrared spectrophotometer (FT-IR) is a tool that is used to identify the chemical bonds present in the compound. The compound isolated and purified from Ethanol leave extract of *T. procumbens* was subjected to FT-IR analysis to identify the chemical bonds.

^{1}H-NMR Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical chemistry technique used in quality control and research for determining the content and purity of a sample as well as its molecular structure. When molecules are placed in a strong magnetic field, the nuclei of some atoms will begin to behave like small magnets. The resonant frequencies of the nuclei are then measured and converted into an NMR spectrum that displays all of the right frequencies as peaks on a graph with that can determine the number of protons present in the compound.

The compound isolated from Ethanol extract of *T. procumbens* was subjected to Proton NMR analysis to identify the number of protons present in the compound.

GC-MS Gas chromatograph mass spectrometers (GC-MS) are for volatile chemical compounds what liquid chromatography (LC) MS systems are to materials in solution: they separate and analyze gas-phase molecules. In LC, compounds are fractionated based on their differential affinity for the separation's mobile (liquid) and stationary phases (column packing material). In GC, heated, volatile compounds are injected into a flowing gas stream and separated as that stream flows through a long hollow tube (sometimes tens of meters in length) coated with a stationary phase material. In GC MS,

the fractionated compounds are then ionized (typically by electron ionization) and injected into the mass spec analyzer, most commonly a single quadrupole (though triple quadrupoles and time-of-flight analyzers are also available). The compound isolated and purified from Ethanol leave extract of *T. procumbens* was subjected for GCMS analysis to know the molecular mass of the isolated compound.

Results and Discussion

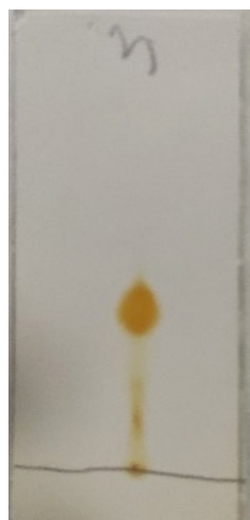
Qualitative analysis of Phytochemicals

After the successful conventional hot Soxhlet extraction of the *T. procumbens* leaves, the preliminary phytochemical study revealed that ethanol extract of *T. procumbens* Linn contains Alkaloids, Flavonoids, Phenols, Oils and Fats and tannins. The list of phytochemicals present is summarized in Table 1. The presence of these phytochemicals in methanol extract of *T. procumbens* was also reported (23).

Table 1: Phytochemical evaluation of ethanol extracts of T. Procumbens

Phytochemicals	Name of the Test	Result
Alkaloids	Wagner's test	Positive
Oils and fats	Saponification test	Positive
Phenols	Ellagic acid test	Positive
Flavonoids	Shinoda test	Positive
Tannin	Ferric Chloride test	Positive

Separation of compounds using PTLC



Qualitative analysis of phytochemicals showed the presence of Oils and fats and these gives different Rf value depending on the solvent used. They were confirmed based on their Rf value, PTLC color in normal and UV light. A combination of solvent such as Dichloromethanol: Methanol (7:1) shows a good results but a combination of solvents such as Toluene:Chloroform:Methanol (4:4:2) shows a better separation than other solvent ratio. The Rf valve obtained for the isolated compound is 0.47%. (Fig 1)

Fig. 1. Separation of isolated compound by PTLC

Separation of compound by Column Chromatography

During the process of separation by Colum chromatography the total 20 fractions of 100mL of each were collected and subjected to TLC, the fraction which is showing same Rf value were merged together and evaporated in desiccator. After complete evaporation a semi solid compound obtained to this further solubility test was carried out to known the solubility of compound. The purified compound was later subjected to its spectral analysis such as FT-IR, ^{1}H-NMR, and GC-MS for identification and structural elucidation.

Pharmacological studies:

Antifungal Activity by cup plate method

The antifungal activity of isolated compound was tested against Candida sp. and Malassezia sp. by well diffusion method. Various concentrations such as 75µg/ml, 150µg/ml and 300µg/ml were prepared and subjected to antifungal activity, after the incubation of petri plates for 48 hours the zone of inhibition was measured from each well of each concentration to both the fungi sp. And the results were illustrated in the Table 2.

Table 2: Antifungal activity of isolated compound

Fungi sp.	Control	Zone of inhibition in Different Concentrations of bioactive compound (cm)		
		75µg/ml	150µg/ml	300µg/ml
Malassezia sp.	5.0	0.0	0.8	1.0
Candida sp.	3.0	0.0	0.5	0.7

Fig. 2. Antifungal activity of isolated compound

The antifungal activity of *T. procumbens* may be due to the presence of many bioactive compounds including phenols, flavonoids, saponins, sterols and fatty acids as reported earlier (24). The bioactive compounds such as 8,3_-dihydroxy-3,7,4_-trimethoxy-6-*O*-ˇ-d-glucopyranosyl flavones, 6,8,3_-trihydroxy-3,7,4_-trimethoxyflavone, puerarin, esculetin, oleanolic acid, betulinic acid, centaurein, bergenin and centaureidin have previously been isolated and characterized from this plant (25). These bioactive compounds may have some role in antifungal activity.

Anti-Inflammatory activity

The in vitro anti-inflammatory activity of isolated compound assessed against denaturation of egg albumin and the results were illustrated in the table 3.

Denaturation of tissue proteins may be the cause behind the production of auto-antigens in certain arthritic diseases. So it may be said that tissue protein denaturation

Table 3: Anti-inflammatory activity of isolated compound

	Bioactive compound	Diclofenc sodium
Blank	0.00	0.00
Test 1	0.63	0.03
Test 2	0.77	0.05
Test 3	1.30	0.09

is a marker for inflammatory and arthritic diseases. (26) Agents that can prevent protein denaturation, therefore, would be possible candidate for anti-inflammatory drug development. With this idea in mind, the *in vitro* test was done as a preliminary screen to check presence of anti-inflammatory property before doing the *in vivo* test. In the present study, the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property of isolated compound from *T. procumbens* with different range of dose concentrations.

The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by both the test compound (Table 3). Diclofenac sodium (at the concentration range of 50-2000 µg/ml) was used as the standard drug, which also exhibited concentration dependent inhibition of protein denaturation (Table 3). This was chosen as a standard NSAID on the basis of previous reports of its use for the same purpose (27, 28). The increased absorbance in both the extracts and the standard drug with respect to control indicates the protein stabilizing activity (denaturation is inhibited) with increased dose.

The essential oils of *T. procumbens*have shown antioxidant activity by reducing the levels of oxidative stress when using the DPPH assay, these essential oils seem to have higher antioxidant activity than ascorbic acid and increasing the concentration of the essential oil seemed to increase the antioxidant power. It is postulated that this characteristic of *T. procumbens* makes it a great candidate for the treatment of inflammation and cancer with less toxic effects (29) but these claims are not properly researched and documented. For example, *T. procumbens*has shown to reduce inflammation when applied as a leaf poultice and it has shown to be effective in the treatment of neuropathic and inflammatory pain in rodent models (30). (Table 3)

Calculations,

Percentage of Inhibition=100 X (vt/Vc-1), Where, vt= absorbance of test sample, vc= absorbance of control. % of inhibition = 100X0.74/1 = 74%.

Clot Lysis activity

Clot lysis is performed to examine whether the isolated compound has the capability of lysing clotted blood, which could be helpful in treating patients who are suffering from blood clotting related disease and disorders. The results of clot Lysis activity are shown in Table 4.

Table 4: Clot lysis activity of isolated compound of T. procumbens

Concentration	Plain tube (g)	Whole blood (g)	Clotted blood (g)	After Plant extract (g)	Weight difference(g)
Negative Control	1.12	1.65	1.38	1.37	0.01
Positive Control	1.12	1.65	1.41	1.19	0.22
25 µg/ml	1.11	1.59	1.35	1.30	0.05
50 µg /ml	1.12	1.62	1.36	1.29	0.07
100 µg /ml	1.10	1.59	1.34	1.22	0.12

A number of studies have been conducted by various researchers to find out the herbs and natural food sources and their supplements having antithrombotic (anticoagulant and antiplatelet) effect and there is evidence that consuming such food leads to prevention of coronary events and stroke (31, 32). Herbal preparations, if taken in appropriate dose, can lead to a better option for curing various ailments. In our thrombolytic assay, the comparison of positive control with negative control clearly demonstrated that clot dissolution does not occur when water was added to the clot. When compared with the clot lysis percentage a significant thrombolytic activity was observed after treating the clots with isolated compound (33).

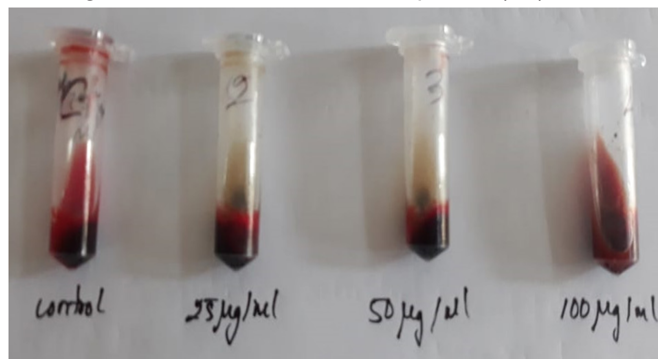


Fig. 3. Clot lysis activity of isolated compound

Identification and structural elucidation of isolated compound by spectral analysis

FT-IR: The FT-IR spectrum was used to identify the functional group of different phytoconstituents based on the peak values in an infrared region of isolated compound from ethanol extract of *T. procumben.* The result of FTIR peak values were shown in Fig.8, The peak at 3292.2 cm¯1 is for O-H bonds of alcohols and phenols (H-bonded hydroxyl group) and carboxylic acids. The peaks at 2981.55cm¯1 are the stretching of C-H bond of methyl and aldehyde group. The peaks at 1636.42 cm¯1 are the stretching of C=O due to the presence of aldehyde group and the peak at 1083.81 cm¯1 is the C-O stretching of alcohol and carboxylic acids. FTIR analysis of *T. procumbens* shows the presence of

alcohols, phenols, carboxylic acids, aldehyde groups.

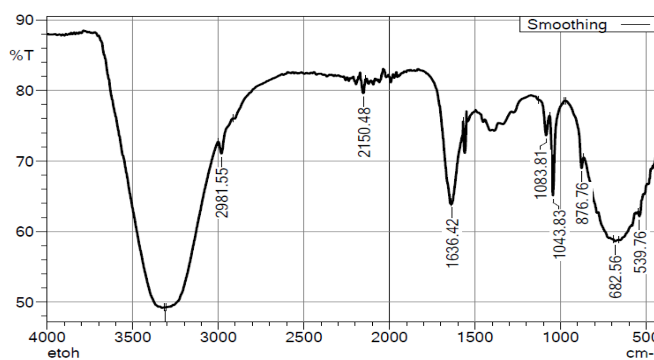


Fig. 4. FT-IR analysis of isolated compound of T. procumbens leaves extract

^{1}H-NMR The NMR spectroscopy analysis of isolated compound from ethanol extract sample was carried out and the results are given in Fig.10. The spectrum distinctly displayed the singlet's at 1-5ppm clearly indicates that the presence of alcoholic compounds (R-OH). The peaks at 1.5 ppm, 2.0ppm, 3.3ppm, 4.5ppm,, and 5.0ppm are indicates the equivalent protons and found to be 18-19 protons present in the compound. There is no peaks found in between 5-8ppm indicates the absence of aromatic compounds in the sample. During the interpreting ^{1}H-NMR spectrum the NMR rules were followed such as chemical and magnetically equivalent of protons and also n-1 (n=number of protons at a peak). The NMR spectrum revealed that sample has alcoholic compound (R-OH) that has 18-19 protons (H), absence of aromatic compound. The identification was further confirmed by available literature data. The similar results were reported (34), in their research explained in detail about determination of fatty acids profile by NMR spectroscopy of both saturated and unsaturated fatty acids. (Figure 5)

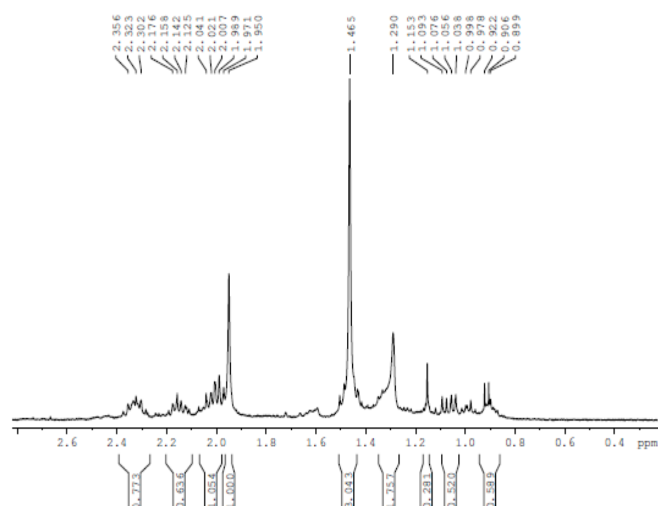


Fig. 5. NMR spectroscopy analysis of purified compound

GC-MS The GCMS spectrum of isolated compound was analyzed in detail and it displayed the molecular ion (M+) peaks at m/z 73, 60, 41 and the sum total of all splitted molecular ions is 174, it approximately equal to the molecular weight of Decanoic acid (saturated fatty acid) is 172.26 and also corresponds to the molecular formula $C_{10}H_{20}O_2$. The correlation of molecular weight and formula was confirmed from the available literature data. The present study results of GCMS analysis of isolated compound can be compared with the GCMS analysis of bioactive fractions of *T. procumbens* reported (35). In their study they were reported more than 20 saturated fatty acids and their different form with structural formula. (Figure 6)

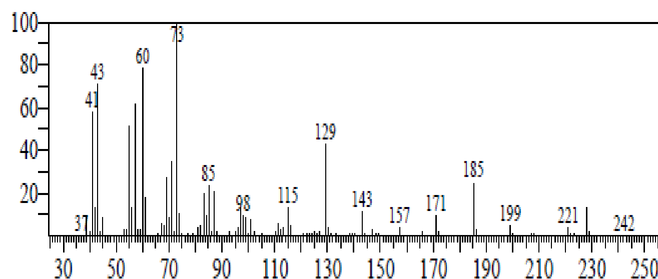


Fig. 6. GC-MS analysis of isolated compound from T. procumbens leaves extract

With reference to the all above spectral data analysis it clearly indicates and correlate that expected bioactive compound from ethanol extract of *T. procumbens* is Decanoic acid (saturated fatty acid) with molecular formula $C_{10}H_{20}O_2$ and structural formula as shown in the fig 7. Other names Caprinic acid; Caprynic acid; Decoic acid; Decylic acid; 1-Nonanecarboxylic acid.

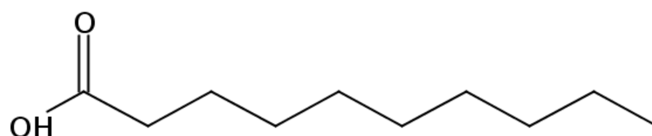


Fig. 7. Showing the structure of Decanoic acid

Conclusion

The present investigation revealed the presence of secondary metabolites such as Alkaloids, Flavonoids, Oils and Fats, Tannins and phenols in the methanol extract of *T. procubens*. The bioactive fraction of methanol extract shown strong antifungal activity and clot Lysis activity but moderate in anti-inflammatory activity. The spectral data analysis clearly indicates that the presence of Decanoic acid (Saturated fatty acid) in the bioactive fraction. The antifungal activity of the active fraction may be attributed to the presence of saturated fatty acid Decanoic acid and their derivatives. Further more detailed study has to be done for the therapeutic activity.

Acknowledgments

The authors are thankful to the Department of Life Sciences, Kristu Jayanti College, for giving an opportunity to undertake this project work and supporting us immensely throughout the duration by providing all

the necessary facilities to carry out this project work.

References

1. Hilliard, O. M., (1977). Compositae in Natal. Pietermaritburg. South Africa: University of Natal Press.

2. Ravikumar, V. Shivashangari, K. S. & Devaki, T. (2005b). Hepatoprotective activity of *Tridax procumbens* against d-galactosamine-lipopolysaccharide-induced hepatitis in rats. Journal of Ethnopharmacology, 101(1-3), 55-60. doi:10.1016/j.jep.2005.03.019.

3. Kethamakka, S. R. P. & Deogade, M. S. (2014). Javanti veda (*Tridax procumbens*) unnoticed medicinal plant by Ayurveda. Journal of Indian System of Medicine, 2(1), 6-20.

4. Srivastava, S. L. Kediyal V. K. Sundriyal R. C. (1984). Screening of floral extracts of some floweriest plants in antifungal activities against *Bipolarize oryzoe*. Journal of Environmental Biology, 5:217–20.

5. Udupa S. L. Udupa, A. L. Kulkarni, D. R. (1991). Influence of *Tridax procumbens* on dead space wound healing. Fitoterapia, 62:146–50.

6. Saraf, S. Dixit, V. K. Tripathi, S. C. Patnaik, G. K. (1992). Hepatoprotective activity of *Tridax procumbens*. Part III. Fitoterapia, 63:414–6.

7. Bhat, R. S. Shankrappa, J. Shivakumar, H. G. (2007). Formulation and evaluation of polyherbal wound treatments. Asian Journal of Pharm Science, 2:11–7.

8. Vilwanathan, R. Shivashangari, K. S. Devak, T. (2005). Hepatoprotective activity of *Tridax procumbens* against dgalactosamine/lipopolysaccharide-induced hepatitis in rats. Journal of Ethnopharmacology, 101:55–60.

9. Bhagwat, D. A. Killedar, S. G. Adnaik, R. S. (2008). Antidiabetic activity of leaf extract of *Tridax procumbens*. International Journal of Green Pharm, 2:126–8.

10. Nia, R. Paper D. H. Essien, E. E. Oladimeji, O. H. Iyadi, K. Franz, G. (2003). Investigation into in-vitro radical scavenging and in-vivo anti-inflammatory potential of *Tridax procumbens*. Niger Journal of Physiology Science, 18:39–43.

11. Mahato, R. B. Chaudhary, R. P. (2005). Ethnomedicinal study and antibacterial activities of selected plants of Palpa district, Nepal. Science World, 3:26–31.

12. Sneha Mundada and Ruchi Shivhare. (2010). Pharmacology of *Tridax procumbens* a Weed, International Journal of PharmTech Research, vol.2, No.2, 1391-1394.

13. Nikita Sanghavi, Rashmi Srivastava and Yashwant Malode. (2014). Isolation and identification of the flavonoid “quercetin” from *Tridax procumbens* L. International journal of pharmaceutical science research, 5(4): 1454-1459.

14. Kokate, C. K. (1994). Practical Pharmacognosy. Vallabh Prakashan, Delhi, Edition 4th, 107-11.

15. Gibbs, R. D. (1974). Chemotaxonomy of Flowering Plants. McGill Queen’s University Press Montreal and London, 1: 160-62.

16. Harbone, J. B. (1973). Phytochemical Methods, 49–188, Chapman and Hall, London, UK.

17. Sofowara, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria.

18. Trease, G. E and Evans W.C. (1985). Pharmacognosy. Bahiv Tinal, London, Edition 17th, 149.

19. Kotze, M. Eloff, J. N. (2002). Extraction of antibacterial compounds from *Combretum microphyllum* (Combretaceae) South African Journal of Botany, 68:62–67.

20. Daoud, A. Malika, D. Bakari, S. Hfaiedh, N. Mnafgui, K. Kadri, A. (2015). Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of date palm pollen (DPP) from two *Tunisian cultivars.* Arab. Journal of Chemistry, 10.1016/07.014.

21. Sharmila, Dharmadeva, Lahiru Sandaruwan Galgamuwa, C. Prasadinie and Nishantha Kumarasinghe. (2018). *In vitro* anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. Ayurveda, 39(4): 239–242.

22. Prasad, S. Kashyap, R. S. Deopujari, J. Y. Purohit, H. J. Taori, G. M. Daginawala, H. F. (2006). Development of an in vitro model to study clot Lysis activity of thrombolytic drugs. Thrombosis Journal, 4:14.

23. Pankaj Kushwaha, Shiv Shankar Yadav, Vigyan Singh and Dwivedi, L. K. (2019) phytochemical screening and GC-MS studies of the methanolic

extract of *Tridax procumbens,* International journal of pharmaceutical science research, 10(5): 2492-2496.

24. Manjamalai, A. Singh, R. S. S. Guruvayoorappan, C. Grace, V. M. B. (2004). Analysis of phytochemical constituents and antimicrobial activity of some medicinal plants in Tamil Nadu, India. Global Journal of Biotech Biochem, 5:120–8.

25. Xu, R. Zhang, J. Yuan, K. (2010). Two new flavones from *Tridax procumbens* Linn. Molecules, 15:6357–64.

26. Dey, P. Chatterjee, P. Chandra, S. Bhattacharya, S. (2011). Comparative *in vitro* evaluation of anti-inflammatory effects of aerial parts and roots from *Mikania scandens*. Journal of Advance Pharm Education Research, 1:271–7.

27. Sarma, D. S. Babu, A. V. Krishna, K. R. (2011). Antioxidant and anti-inflammatory activities of *Thespesia populnea* L. International journal of research in pharmacy, 1:674–6.

28. Kamble, P. S. Palatty, P. L. Kamble, S. (2012). Comparative study of anti-inflammatory effects of rosiglitazone and pioglitazone with diclofenac sodium in carageenan induced rat hind paw oedema. International journal of basics applied medical sciences, 2:1–7.

29. Manjamalai, A & Grace, V. M. B. (2004). Effect of essential oil of *Tridax procumbens L* on in-vivo antioxidant level in cancer model and in-vitro free radical scavenging activity. International Journal of Pharmaceutical Analysis, *37*(10), 261-271.

30. Sawant, S. Chine, V. Kalange, A. Joshi, P. Gawali, V. Praharaj, S. N. Jangme, C & Siddiqui, M. (2014). Evaluation of lyophilized extract of leaves of *Tridax procumbens* L. In rodent models of inflammatory and neuropathic pain. Orient Pharm. Exp. Med., *14*(2), 163-167.

31. Nyarko, A. A and Addy, M. E. (1990). “Effects of aqueous extract of *Adenia cissampeloides* on blood pressure and serum analyte of hypertensive patients,” Phytotherapy Research, 4, 1, pp. 25–28.

32. Gillman, M. W. Cupples, L. A. Gagnon, D. (1995). “Protective effect of fruits and vegetables on development of stroke in men,” The Journal of the American Medical Association, 273, no. 14, 1113–1117.

33. Bazzano, L. A. He, J. Ogden, L. G. (2002). Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study, The American Journal of Clinical Nutrition, 76, 1, 93–99.

34. Gerhard Knothe and James A. Kenar**.** (2004). Determination of the fatty acid profile by 1H-NMR spectroscopy, European Journal of Lipid Science Technology*,* 106**,** 88–96.

35. Policegoudra, R. S. Chattopadhyay, P. Aradhya, S. M. Shivaswamy, R. Singh, L. V. Veer, V. (2014). Inhibitory effect of *Tridax procumbens L* against human skin pathogens. Journal of herbal medicine, 4. 83–88.