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A Comprehensive Review on Plant *Tridax procumbens* and Analytical Methods for its Active Constituents

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Abstract: *Tridax procumbens* is a plant belonging the family *liliaceae* or *asteraceae*, generally used in traditional medicines. Plants have long been used as a source of medication, and India's healthcare system heavily relies on them. In the current study, the various phytochemicals found in *Tridax procumbens* one of the medicinally significant plants commonly found in subtropical countries and growing primarily at waste places and roadsides throughout India during the rainy season were evaluated using chromatography. Flowers have a capitulum inflorescence, are tubular in shape, and are yellow in colour. This has disc florets with a basal placentation and ray florets, two different types of flowers. It shows hepatoprotective, antioxidant, anti-inflammatory, anti-arthritic and wound healing activity containing different phytoconstituents such as Alkaloids, flavonoids, carotenoids, iso-sitosterol, luteolin, quercetin, oxoester, lauric acid, myristic, palmitic, arachidic, linoleic, and tannin. In this review we have studied about different analytical techniques of *Tridax procumbens* as HPLC, TLC, high resolution liquid chromatography-mass spectroscopy.

I. INTRODUCTION

The two species of the genus *Tridax procumbens*, *Tridax balbisioides* and *Tridax trilobata*, are Asteraceae or Compositae plants. It is a Central American annual or perennial weed that can be found all over India, but is most common in the states of Maharashtra, Madhya Pradesh, and Chhattisgarh. Lonesome, protracted stalked, heterogamous, bisexual, blended yellow blooms having white flower with extremely haired heads, rough serrated, petiolate, leaves that are oval or unsubdivided, it frequently roots at nodes. The seeds are used to decrease bleeding, while the leaves provide antisecretory, insecticidal, wound-healing, and hypotensive properties.¹

II. MORPHOLOGY AND CYTOPATHOLOGY

The semi-prostrate annual creeper herb *T. procumbens* has stems that reach heights of 30 to 50 cm, are rooted at nodes, and is sparsely hairy. The opposite, obtuse, pointy, fleshy, glabrous, exstipulate, lanceolate to ovate in shape, 3–7 cm long, irregularly serrated along the border with a wedge-shaped base, short petioled, and hairy on both sides leaves have these characteristics. The leaves contain distinct upper and lower faces have a thick cuticle covering their single-layered epidermis on both surfaces. Single-layered, elongated cells make up the lower epidermis or tightly organised, whereas the upper epidermis has a single layered, multicellular covering trichome.² Crystals of calcium oxalate can be seen in the xylem vessel. Vascular bundles have a concentric structure. Meristele is made up of a single auxiliary vascular bundle that is positioned in the centre and is encircled by some parenchymatous cells.² Flowers have a capitulum inflorescence, are tubular in shape, and are yellow in colour.³ This has disc florets with a basal placentation and ray florets, two different types of flowers. The fruit, an achene that is rigid and covered in stiff hairs, has a white pappus that is feathery and plume-like at one end that aids in airborne distribution.⁴ The heads are diverse and have long peduncles that can grow as tall as 2 feet. The trifid, female ray florets are always pale-yellow in hue with a ligulate corolla.⁵ In the presence of 58 to 78% light, at warmer temperatures (35/25 and 30/20 °C), *Tridax procumbens* seeds germinate. They are extremely vulnerable to high salt content and water stress. In gametes, there are 18 (haploid) and 36 (diploid) exist chromosomes.⁵ Stems are stretched out and seeds are produced throughout the propagation process.⁴

III. MEDICINAL USES

Aqueous leaf extract has a heart-healthy effect and considerably lowers blood pressure and heart rate. Similar to aspirin and ibuprofen, lyophilized aqueous leaf extract demonstrated anti-inflammatory activity. Aerial portions in their entirety offer hepatoprotective and antidiarrheal properties. Fungi, bacteria, and protozoa are all susceptible to *Tridax procumbens*. Leaf juice aids in the healing of open wounds. To examine various types of bleeding, seeds are employed. As an immunomodulator, complete aerial portion aqueous extract is employed. Even when put in a mineral basis, dry extract exhibited antibacterial action.⁶

IV. ACTIVITY OF TRIDAX PROCUMBENS

The activity of Tridax procumbens are given as below:

A. Antioxidant Activity

By activating the transcription factor NF-B, the oxygen free radicals produced by phagocytes cause the release of pro-inflammatory cytokines and activation of COX-2. This starts a cascade of tissue damage that has to be prevented. Antioxidant properties are seen in Tridax procumbens. This was verified using the 2, 2'-azino-bis (3-ethyl benzothiazoline-6-sulphonic acid) ABTS and DPPH (2, 2-diphenyl-picrylhydrazyl hydrate) methods. With IC₅₀ values of 37.39 g/ml for hepatoprotective properties, the chloroform and ethyl acetate fractions of the ethanol extract demonstrated the greatest levels of DPPH technique. Moreover, the 2, 2-diphenylpicrylhydrazyl (DPPH) technique revealed antioxidant activity in the methanol extract. The extracts' flavonoids and alkaloids are primarily accountable for the activity.⁷

B. Hepatoprotective Property

The body's primary organ for detoxification is the liver. Enzymes involved in the detoxification process are found in the liver. The enzymes are released into the blood stream by any damage to the liver cells.⁸ The severity and type of hepatocellular injury are revealed by the estimation of the serum marker enzyme.

There was hepatoprotective action in Tridax procumbens. As evidenced by the decline in enzyme markers like aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase, gamma glutamyl transferase, and bilirubin in the serum of the animal model, an aerial part of Tridax extract in chloroform significantly reduced lipopolysaccharide and D-galactosamine-induced hepatitis in rats. The extract thereby reduces liver damage and initiates the regeneration of parenchymal cells in the liver.^{4,7,9} Hepatitis caused by d-Gal N/LPS, carbon tetrachloride, medicine (paracetamol), and oxidative stress might be prevented by Tridax procumbens extracts in aqueous, ethanolic, and chloroform.¹⁰ Comparable findings were also shown when T. procumbens extract was combined with chloroquine. In the rat model of d-Gal N/LPS-induced hepatotoxicity, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), glutathione-s-transferase (GST), glutathione, vitamin C, and vitamin E concentrations were all decreased as a result of increased levels of TBARS (thiobarbituric acid reactive substance). The body's non-enzymatic, detoxifying, and antioxidant defence mechanisms are among the decreasing components. After ten days of oral administration of T. procumbens chloroform extracts, this imbalance returned to normal.

The centre zone of the perivenular area lacked cellular necrosis and an inflammatory infiltration, according to histological examinations, which provided additional evidence for this. Tridax procumbens aqueous extract reduced chloroquine-induced hepatotoxicity by preserving the hepatocellular membrane's structural integrity. In a rat model, the methanolic extract decreases hepatotoxicity marker enzymes while preserving total bilirubin count, direct bilirubin count, hydroxyproline, lymphocyte infiltration, and bile duct proliferation, avoiding bile duct ligation-induced liver fibrosis. Because of its saponins and flavonoids, the extract may have hepatoprotective qualities.¹¹

C. Anti-inflammatory Activity

By inhibiting the effects of inflammatory mediators such histamine, serotonin, bradykinin, and prostaglandins, the extracts in water, ethyl acetate, methanol, and ethanol demonstrated considerable anti-inflammatory potential. The solvent extracts' three newly discovered active substances, bergenin, centaureidin, and centaurein, all inhibited the COX-1 and COX-2 enzymes. Analgesic and anti-inflammatory properties are included in the flavonoid compound quercetin. In a model of chronic constriction injury (CCI)-induced neuropathic pain, this may also be the cause of the regulation of inflammatory pain and anti-allodynic action.^{12,13}

D. Anti-Arthritic Activity

Inflammation causes arthritis characterised by joint deterioration in a joint or many joints. In contrast to the conventional medicine, indomethacin, ethanolic Tridax procumbens extract demonstrated considerable anti-arthritic activity in a rat model produced by Freund's Complete Adjuvant. An increase in body weight, RBC count, and haemoglobin level was evaluated, ESR levels, WBC counts, and pannus formation all declined, and bone degeneration. Articular cartilage loss is a defining feature of rheumatoid arthritis, This results in diminished joint spaces as a result of significant soft tissue swelling brought on by a variety of pathogenic causes, Additionally, bone resorption was reduced when an ethanolic extract of Tridax procumbens was administered, demonstrating the extract's anti-arthritic properties.¹⁴

E. Wound Healing

In Indian traditional medicine, leaf extract is frequently used topically to open wounds to stop bleeding and hasten healing. Increased levels of lysyl oxidase and hexosamine in the plant extract showed wound healing activity in rats. These compounds are known to promote collagen crosslinking throughout the healing process to stabilise collagen strands. Furthermore, the extract enhances the production of glycosamine glycans (GAGs), the primary constituents of ECMs in granulation tissue, both at the mRNA and protein levels. Through interactions with the extracellular matrix, soluble proteins, dermal and epidermal cells, as well as cytokines and growth hormones that regulate the angiogenesis processes, *Tridax procumbens* aids healing of wounds. The ethanolic extract shown notable wound healing effectiveness in a gel-based formulation. The healing of excision wounds is indirectly influenced by corticotropic actions of *Tridax procumbens* extract. This boosts the tensile strength of collagen fibres as well as the pace of epithelialization. When used topically, the leaf extract is said to reduce bleeding.^{15,16,17,18,19}

V. TRADITIONAL USES

Traditional and complementary medicine are increasingly being acknowledged by many countries as an integrated method of treating illness (WHO, 2013). The usage of plants for therapeutic purposes may have begun some 60,000 years ago, during the Middle Palaeolithic period. *T. procumbens* is distributed all around the world.²⁰ In Central America to treat hepatopathies, colds, inflammation, anaemia, and other conditions. *Tridax procumbens* is a plant that is utilised in Guatemala as a natural antibacterial, antifungal, and antiviral treatment as well as for vaginitis, stomach discomfort, diarrhoea, mucosal inflammations, and skin infections. Healing and bleeding-stopping properties of the leaf juice. A research conducted in Chiquimula, Guatemala, discovered that breastfeeding pregnant women with anaemia might improve their symptoms by using *Tridax*. In addition to being used to treat diabetes and high blood pressure, this species is also utilised to treat digestive and respiratory conditions. The whole plant is used to treat protozoal illnesses in Guatemala.^{21,22} Among the illnesses include dysentery, leishmaniasis, and malaria. Aqueous *T. procumbens* extract effectively combats chloroquine-resistant *P. falciparum* parasites. The whole plant is used in Nigeria to treat epilepsy, stomachaches, backaches, typhoid fever, cough, and other ailments.^{23,24}

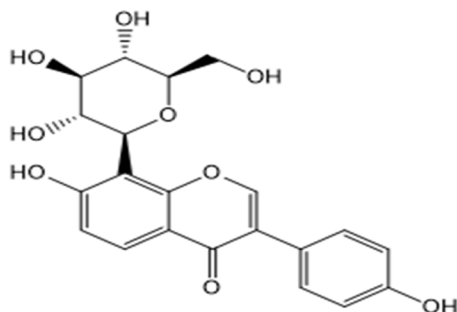
VI. CHEMICAL COMPOSITION

At 88.30% and 90.05%, respectively, the stem and leaves of *T. procumbens* are very wet. The stem and leaf both contain a significant amount of protein, having a dry weight of 37.34% and 4.38% wet weight, respectively. The leaf weighs 0.6 wet weight and 6.03% dry weight or (5.10% wet weight) 51.26% dry weight, Compared to the 0.85% dry weight and 0.1 wet weight of the stem, respectively, and their respective weights of 41.03% and 4.80%, total lipid or carbohydrate contents, respectively. The leaf has 6.13% dry weight of crude fibre and 0.61% wet weight, compared to the dry weight (16.41%) and wet weight (1.92%) of the stem. With a moisture level of 88.30%, It's really damp within *T. procumbens*. The leaf of *T. procumbens* contains around 397.59 Kcal (39.56 Kcal wet weight) and the stem contains about 321.54 Kcal (37.62 Kcal wet weight) per 100 g. The plant contains significant amounts of the trace elements magnesium, phosphorus, potassium, selenium, and Iron, copper, manganese, sodium, zinc, and calcium are included in the list. The aqueous extract contains a variety of phytochemicals, including steroids, catechins and flavones in flavonoids, alkaloids, carotenoids, saponins, and tannins. Ethyl acetate is used in organic solvent extraction to extract the flavonoids centaureidin and centaurein as well as bergenin. Among the 2° metabolites identified were fumaric acid, sterols, glucoluteolin, isoquercetin, quercetin, and luteolin.²⁵

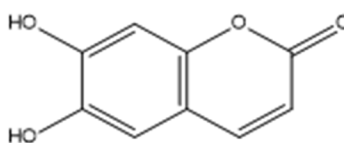
A. Chemical Constituent

Tridax procumbens, the plant, a variety of active chemical constituents were discovered and reported. Alkaloids, flavonoids, carotenoids, iso-sitosterol, luteolin, quercetin, oxoester, lauric acid, myristic, palmitic, arachidic, linoleic, and tannin are some examples of plant compounds, among others, are examples of compounds. Earlier studies found the presence of quercetin, beta-sitosterol, luteolin, and glucoluteolin. The aerial sections were also said to contain linolenic acid. Two water-soluble polysaccharides with - (1->6) are WSTP IA and WSTP IB.-The plant's leaves were also used to purify the main chain of DGalactan. *Tridax procumbens*' lipid components and the identification of certain sterols by GC-MS were both reported. A novel flavonoid known as "Procumbenetin" has been determined to be 3, 6-dimethoxy-5, 7, 2', 3', and 4' pentahydroxyflavone 7-O-glucopyranoside. *T. procumbens* leaves have been shown to contain calcium, magnesium, potassium, sodium, and selenium as well as other minerals. *T. procumbens* has been shown to be a viable source of provitamin A (carotenoids) for the general public as well as an excellent plant protein source with potassium supplement. In addition to bis bithiophene, four novel terpenoids from *T. procumbens* were reported: Lupeol, beta-amyrone, taraxasteryl acetate, and oleanolic acid.

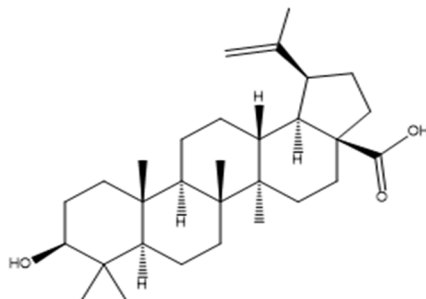
Tridax procumbens Linn. yielded four known compounds puerarin and 8,3'-dihydroxy-3,7,4'-trimethoxy-6-O--D glucopyranosyl flavone and 6,8,3'-trihydroxy-3,7,4'-trioxyflavone are two new flavones. Betulinic acid, esculetin, and oleanolic acid.²⁶



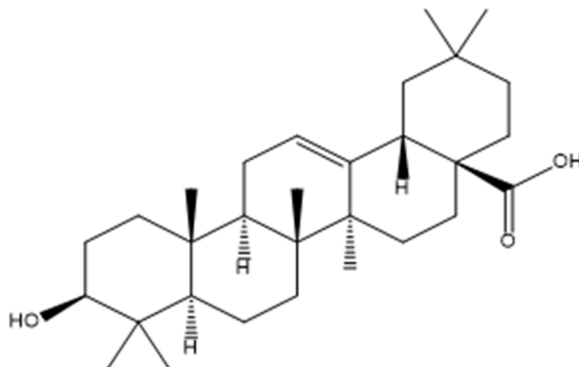
Puerarin²⁶



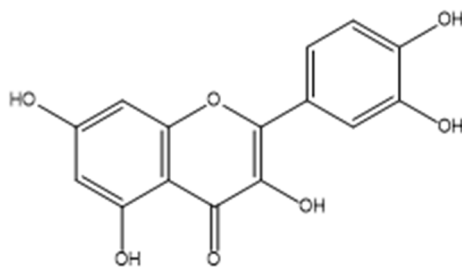
Esculetin



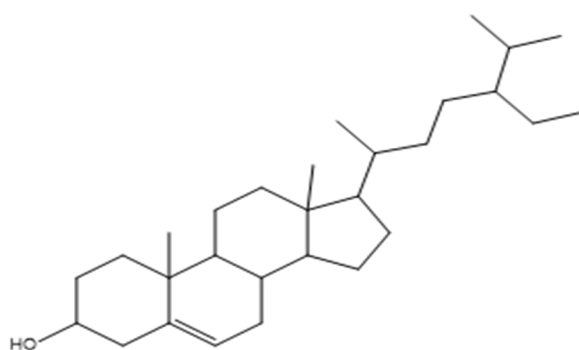
Betulinic acid



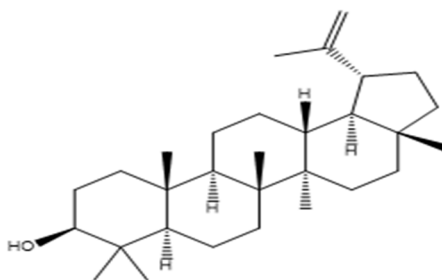
Oleanolic acid



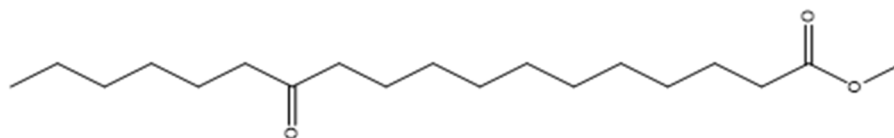
Luteolin



Beta-sitosterol



Luperol



Methyl 14-oxooctadecanoate

VII. ANALYTICAL METHOD FOR TRIDAX PROCUMBENS

- 1) **HPLC Analysis:** The extracted and purified substance from *Tridax procumbens* leaves was examined for conformance and purity. To check the purity of the chemicals, the extracts from the leaf, stem, root, and flower were prepared for HPLC (High performance liquid chromatography). In the HPLC, both mobile and stationary phases are employed to check the compound's purity. The mobile phase is often a single gradient or a binary gradient. It is clear from the data that is currently accessible that the mobility phase utilised is depicted in the images themselves. Since the sample's measurement is less than 400 nm, the D2 lamp is being employed as the detector.²⁷
- 2) **Thin layer chromatography of extract TRIDAX PROCUMBENS:** TLC was used to create the qualitative analysis. Thin layer chromatography has been used to separate the extract's constituent components. For the *Tridax procumbens* extract, 30:15:30:25 chloroform, ethyl acetate, methanol, and butanol were utilised. In a hot air oven set at 1100 C for 15 minutes, silica gel plates were made and dried. The test sample was laid out on a TLC plate, and the plate was then passed through a solvent system. The plate was removed, visualised under a UV transilluminator, and the Rf values for the various bands were measured after running the solvent front up to one-third sections.²⁸
- 3) **Preparative HPLC:** Preparative high-performance liquid chromatography was used to further separate the bioactive chemicals with the bioactivity to generate AgNPs that were isolated from the crude extract by silica gel chromatography. A high-performance liquid chromatography system (Agilent Technologies) was used for the separation, which has outstanding solvent supply control thanks to its prominent pump, high-precision dual-plunger design, and forced check valve. A reverse-phase C18 column (150 mm long, 21.2 mm wide, 5 m thick, 100 pore size, made of spherical fully porous ultrapure silica) was employed. With a mobile phase of methanol and acetonitrile (95:5), an injection volume of 10 l, and a flow rate of 1.0 mL/min, all analyses were carried out at room temperature. By using a silica gel chromatograph, the bioactive components were isolated from the crude extract. A multiwavelength UV detector was used to measure the column effluent at wavelengths of 190 and 220 nm. Fractions were collected for additional investigation into the production of AgNPs and their antibacterial properties. The production of AgNPs was examined in the obtained fractions. The AgNP-producing fractions were chosen, and the microdilution assay was used to assess their antibacterial efficacy against *Shigella* spp. Using HRLC-MS (high-resolution liquid chromatography-mass spectroscopy), the effective AgNPs were examined to look for reducing and capping agents.²⁹
- 4) **High-Resolution Liquid Chromatography-Mass Spectroscopy:** Agilent Technologies employed a dual AJS ESI ion source LC/MS coupled to a high-performance liquid chromatography (HPLC) system for the analysis. Water and acetonitrile were utilised as the solvent system for HPLC at a flow rate of 0.3 ml/min at 1200 pressure at room temperature. The TOF/Q-TOF mass spectrometer's MS system (a solvent system) received an injection of 3 l of eluent at a flow rate of 0.3 ml/min, 1200 pressure, and room temperature. The run time was set at 1 mL/1 minute. A 20 uL injection volume is given to the stationary phase column. The gradient software was developed, and the peak analysis was computed, by looking at the graph and comparing the created chromatogram with that of the pre-existing data.²⁹

VIII. CONCLUSION

It is a weed known as *Tridax procumbens* that is native to tropical America and has naturalised in tropical Africa, Asia, and Australia.

It is a Central American annual or perennial weed that can be found all over India, but is most common in the states of Maharashtra, Madhya Pradesh, and Chhattisgarh. Lonesome, protracted stalked, heterogamous, bisexual, blended yellow blooms having white flower with extremely haired heads, rough serrated, petiolate, leaves that are oval or unsubdivided, it frequently roots at nodes. This plant is widely used, and every portion of it possesses remarkable pharmacological properties.

The research that has been conducted so far on its pharmacological effects, such as hepatoprotective effect, immunomodulating property, promising wound healing activity, antidiabetic, hypotensive effect, antimicrobial, insect repellent activity, anti-inflammatory and antioxidant, bronchial catarrh, dysentery, and diarrhoea, also prevents hair loss and promotes hair growth. The study included that *Tridax procumbens* contain different phytochemical as Pueranin, Esculetin, Betulinic acid, oleanolic acid, Luteolin, Beta-sitosterol etc. Also the study also shows that HPLC, high resolution liquid chromatography, TLC and preparative HPLC are the different analytical method for the estimation of *Tridax procumbens*.

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