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A comprehensive review on Phytochemical Investigation of *Tridaxprocumbens*

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ABSTRACT

Indigenous tribes traditional medical systems heavily rely on *Tridaxprocumbens*, which they have used for generations to harness its healing properties for different health issues.Although it originated in tropical America and has subsequently expanded to tropical,subtropical and temperate climates worldwide. It is widely distributed in India.This little perennial herb has short,hairy,blade- like leaves and is amember of Compositae or Asteraceae family. The extracts of*T.procumbens*obtained through ethanol exhibited notable anti-inflammatory properties by obstructing histamine, serotonin, bradykinin and prostaglandins which are inflammatory mediators.The phytochemical analysis indicated the existence of flavonoids,alkaloids,carotenoids,fumaric acid,saponin and tannins.The primary focus of remedies derived from *Tridaxprocumbens* has been well-documented anti-inflammatory properties. This plant contains various flavonoids, with Quercetin being notable for its anti-inflammatory effects.Flavonoids are a class of polyphenolic secondary metabolite found in plants.Exudate volume, leukocyte migration, edema fluid, granuloma tissue, and γ-glutamyltranspeptidase have all been demonstrated to be greatly reduced by *Tridaxprocumbens* extracts, indicating the plant's potent anti-inflammatory properties. Identification tests indicate that the flavonoids present are primarily concentrated in the leaves, with Quercetin being the predominant flavonoid exhibiting anti-inflammatory properties.Various identification tests for flavonoids were performed. Some spots were identified by Thin layer chromatography and paper chromatography which confirms the presence of compounds(mainly flavonoids) in the fractions obtained from ethanolic extract of leaves of *Tridax procumbens.*

Key words: Anti-inflammatory activity, flavonoids, protein denaturation,*Tridax procumbens*

INTRODUCTION

Tridax procumbens commonly referred to as Coat Buttons or Tridax Daisy, is a member of the Asteraceae family. In the region of West Bengal, it is identified as Tridhara or Bishalyakarani [1]. This plant is characterized as a small, semi-prostrate, herbaceous creeper that can be either annual or perennial, featuring short, hairy, blade-like leaves. The corolla exhibits a yellow hue. The stem can reach heights of 20-60 cm, is branched, sparsely hairy, and capable of rooting at the nodes. The leaves are simple, arranged oppositely, stipulate, and can be described as lanceolate or ovate, measuring 4-8 cm in length with a toothed margin and a wedge-shaped base, possessing short petioles and hair on both surfaces. The flowers are tubular, yellow, and hairy, forming a capitulum inflorescence. The plant produces two types of flowers: ray florets and disc florets [2]. Investigations into the phytochemical constituents and antioxidant properties of this species have been conducted [3, 4, 5], and the findings have contributed to the understanding of its medicinal applications [6, 7]. Previous studies have identified the presence of compounds such as dexamethasone, luteolin, glucotureolin, β-sitosterol, flavone, glycoside, and quercetin within this plant [8, 9, 10]. It has been recognized for its therapeutic potential in treating various conditions, including wound healing [11], dysentery [12], epilepsy, malaria [13], stomachache, diarrhea, hypertension, diabetes [14], hemorrhage, and metabolic syndrome [15]. Additionally, it exhibits insecticidal, antiseptic, parasiticidal, and hepatoprotective properties, along with a significant depressant effect on respiration [16, 17, 18]. This plant is also well-regarded in Ayurvedic medicine for its efficacy in addressing liver disorders, gastritis, and heartburn [19]. Furthermore, it serves as a bio-absorbent for the removal of toxic Cr (VI) from industrial wastewater [20].One well-known example is *Tridaxprocumbens*, which the locals call "Ghamara" and which means "coat buttons" in English. This herb is used by some Ayurvedic practitioners in placeof "Bhringraj"[21].

Synonym	
English	Coat buttons[22,23,24]
Hindi	Ghamra
Sanskrit:	Jayanti Veda ,Kumminnippacha
English	Coat buttons, Tridax Daisy, Mexican Daisy
Oriya:	BishalyaKarani
Telugu	GaddiChemanthi
Tamil:	ettukayathalai, Thatha
Bengali	Tridhara
Marathi:	Kambarmodi, jakhamjudi and tantani
US	Tridax daisy
Nigeria	jayanti,vettukkaaya-thala[25]
Kannada	Jayanthi

Table 1: Synonyms of *Tridax Procumbens*[26]

Taxonomical classification	
Kingdom	Plantae
Subkingdom	Tracheobionta
Division Spermatophyta	Division Spermatophyta
Subdivision	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Asterales
Family	Asteraceae
Genus	Tridax
Species	procumbens

Table 2:Taxonomical classification of *Tridax Procumbens*[27]

Fig.1:*Tridax procumbens*

CHEMICAL COMPOSITIONS

Plant part	Chemical constituents
Leaves	Flavonoids,Phenols,Amino acids , phlobatannins, Diterpenes, Coumarins,Saponins,Steroids,Anthocyanin,Alkaloids,Propenes,Carbohydrates,Cardiac glycosides[28]
Flowers	Steroidal saponins,β-Sitosterol-3-O-β-D-xylopyranoside,Luteolin,glucoluteolin,quercetin,isoquercetin[29]
Aerial parts	Phytosterols,beta-sitosterol,stigmasterol,campesterol,triterpene,beta-amyrin,9,12-octadecadienoic acid ethyl ester(18.04%)5-cholestane(12.42%),cholestaneglycosides,rhamnosides, octadecenoic acid ethyl ester(4.72%),hexadecanoic acid ethyl ester(4.86%)[30,31]
Whole plant	Luteolin, -amyrin, -amyron, lupeol, triacontanol, fucosterol, campasterol, stigma sterol, besides arachidic acid, lauric acid, palmatic acid,flavones , quercitin, Alkaloids, tannins, flavonoids, saponins, and phenolic compounds[32,33]

Table 3: Phytochemicals present in different parts of *Tridaxprocumbens*

Distribution:

T. procumbens is primarily native to Central America; however, it is now distributed across tropical and subtropical regions globally. It commonly grows alongside annual crops, as well as in various environments such as roadsides, pastures, fallow land, dikes, railroads, riverbanks, meadows, dunes, and areas of waste.[34].

Collection:

The plants are collected randomly from local area of PimpalgaonBaswant, Maharashtra (India).

Extract preparation :

a) Plant componentsincluding leaves were detached from the plant and thoroughly rinsed with distilled water.
b) Subsequently, these cleaned plant materials were dried in the shade.
c) The plant materials were ground into a fine powder using a mechanical grinder once they had completely dried, and they were subsequently kept in airtight containers.
d) Ethanolic extract were prepared by dissolving 10% (w/v) of the powdered material in the respective solvents.
e) The extraction process lasted for 48 hours, after which the solvents were removed using a rotary evaporator.
f) For additional examination, the leftover extracts were then redissolved in dimethyl sulfoxide (DMSO).

Fig. 2: Drying

Fig.3: *Tridax procumbens* powder

Fig.4: Extraction

Anti-inflammatory action of *Tridaxprocumbens:*

Inflammation:

Inflammation represents a natural and intricate biological reaction of the body to harmful agents, including pathogens, damaged cells, or irritants.This reaction is a defence mechanism used to get rid of the negative stimuli and encourage recovery.

Types of Inflammation:

1. Acute Inflammation:

- It is a rapid, short-term response that occurs immediately following tissue injury or infection.

- It is marked by classic symptoms such as redness, warmth, swelling (edema), pain, and impaired function.

-Typically, acute inflammation resolves once the harmful stimuli are eliminated, allowing for the initiation of tissue repair[35].

2. Chronic Inflammation:

- It is a prolonged and sustained response that may persist for weeks, months, or even years.

- This type of inflammation can arise from ongoing infections, autoimmune conditions, or extended exposure to irritants.

- Chronic inflammation has the potential to cause tissue damage and may play a role in the development of various diseases, including rheumatoid arthritis, atherosclerosis, and certain cancers.

Mechanisms of Inflammation:

1. Vasodilation and Enhanced Permeability:

• In response to inflammatory agents such as histamine and prostaglandins, blood vessels undergo dilation (vasodilation) and exhibit increased permeability, facilitating the movement of immune cells and fluids from the bloodstream into surrounding tissues.

2. Recruitment of Leukocytes:

• White blood cells, particularly neutrophils and macrophages, are drawn to the area of inflammation to fight off pathogens and eliminate cellular debris.

• Chemotactic agents guide leukocytes to move towards the site of inflammation.

3. Phagocytosis:

• Phagocytic cells, including neutrophils and macrophages, actively engulf and eliminate foreign pathogens, dead cells and debris through a process known as phagocytosis.

4. Release of Inflammatory Mediators:

• Various molecules, such as cytokines, chemokines, prostaglandins, and leukotrienes, are released during the inflammatory response.

• These mediators play a crucial role in regulating immune cell activation, the severity of inflammation, and the processes involved in tissue repair.[36]

Regulation of Inflammation:

1. Resolution Pathways:

• Inflammation is carefully regulated by specialized pro-resolving mediators (SPMs), which aid in ending inflammation and healing tissues.

•Various SPMs, such as lipoxins, resolvins, protectins, and maresins, are essential in finalizing the inflammatory response..

2. Anti-inflammatory Mechanisms:

• Various mechanisms exist to counteract inflammation, thereby preventing excessive damage to tissues.

• Anti-inflammatory cytokines, such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β), serve to inhibit pro-inflammatory pathways.

• Endogenous inhibitors, including glucocorticoids, modulate the activity of inflammatory mediators.

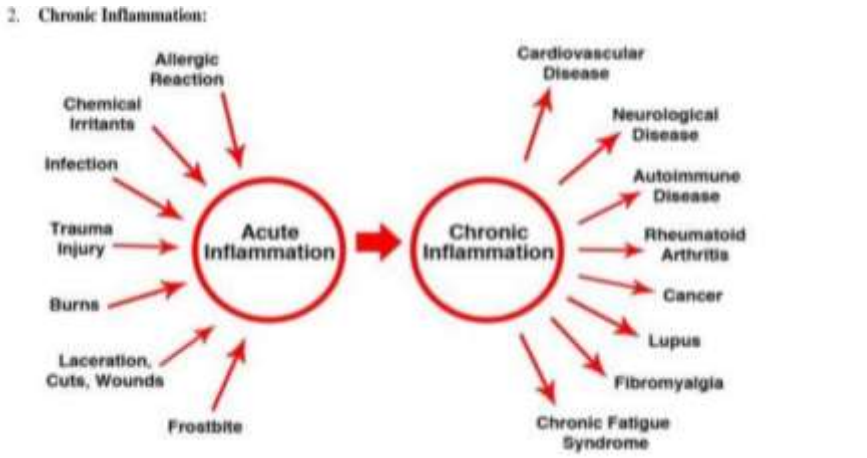

Fig.5: Inflammation

Determination of anti-inflammatory activity

• Anti-inflammatory activity :

The extracts of *T. procumbens* obtained throughethanol method exhibited notable anti-inflammatory properties by obstructing histamine, serotonin, bradykinin, and prostaglandins which are inflammatory mediators[37,38]. Among the active constituents isolated from these solvent extracts, Bergenin, Centaureidinand Centaureinwere identified as inhibitors of COX-1 and COX-2 enzymes. Additionally, the flavonoid Quercetin has been linked to both analgesic and anti-inflammatory effects, potentially contributing to the alleviation of inflammatory pain and demonstrating an anti-allodynic effect in a neuropathic pain model induced by chronic constriction injury (CCI)[39,40].

TEST FOR FLAVONOIDS

Flavonoids possess conjugated aromatic systems, which result in significant absorption bands within the visible and ultraviolet regions of the spectrum. For instance, the principal maxima are observed between 470 nm and 560 nm, while a subsidiary maximum is noted at 275 nm, indicating the presence of anthocyanins. The color characteristics of flavonoids under visible and UV light also facilitate their identification. For example, most flavonol glycosides exhibit a very pale yellow hue in visible light, whereas they appear dark brown in UV light when examined alone, and light yellow to yellow-brown in the presence of ammonia.

(a) Shinoda Test: To dry powder or extract, add 5 ml 95% ethanol/t-butyl alcohol, few drops conc. HCl and 0.5 g magnesium turnings. Orange, pink, red to purple colour appears (flavonols, dihydro derivatives and xanthenes).

Add t-butyl alcohol before adding the acid to avoid accidents from a violent reaction and to dissolve the coloured compounds into the upper phase. By using zinc instead of magnesium, only flavanols give a deep red to magenta colour while flavanones and flavonols give weak pink to magnetic colours or no colour.

b) Sulphuric Acid Test : On addition of sulphuric acid (66% or 80 %) flavones and flavonolsdissolve into it and give a deep yellow solution. Chalcones and aurones give red or red-Dluish solutions. Flavanes give orange to red colours.

c)Alkaline Reagent Test (NaOH Test):

1. Create a solution of the plant extract using methanol.

2. Introduce several drops of a 10% sodium hydroxide (NaOH) solution.

3. The appearance of a yellow color or fluorescence when exposed to UV light suggests the flavonoids presence.

d) Lead Acetate Test:

1. Create a solution of the plant extract in methanol.

2.Add several drops of lead acetate solution

3. The emergence of a yellow precipitate confirms the presence of flavonoids.

e) Ammonia Test:

1. Formulate a solution of the plant extract in methanol.

2. Add several drops of concentrated ammonia solution.

3. The appearance of a yellow color or the development of a yellow tint on filter paper after drying indicates the flavonoids presence.

f) Ferric Chloride Test:

1. Prepare a solution of the plant extract in methanol.

2. Add a few drops of a 10% ferric chloride ($FeCl_3$) solution.

3. The formation of a bluish-green or brown coloration indicates the flavonoids presence.

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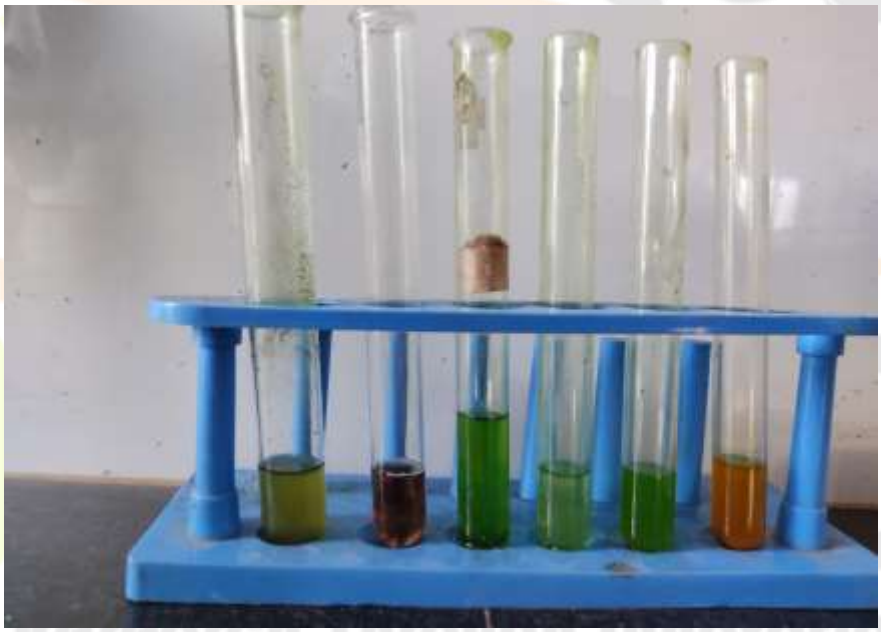

Fig.6: Tests for flavonoids

Identification tests of flavonoids :

Test	Procedure	Observation
Shinoda test	Sample + conc.HCl + Mg	Pink or red colour observed.
Sulphuric acid test	Sample + H_2SO_4	Deep Yellow solution observed.
Alkaline reagent test	Sample + 10% NaOH solution	Yellow colour or fluorescence observed.
Lead acetate test	Sample + few drops of Lead acetate solution	Yellow precipitate observed.
Ammonia test	Sample+ few drops of conc.ammonia solution	Yellow colour observed.
Ferric chloride test	Sample+ 10% $FeCl_3$ solution	Bluish green or brown coloration observed .

Thin Layer Chromatography

TLC technique was used to determine the presence of flavonoids in the extract.

1) Stationery phase: Silica gel

2)Mobile phase:

Toluene : ethyl acetate: formic acid

5 : 4 : 0.2

Saturation time: 15 min

a. The extract was run on silica gel G plate.
b. Visualization was performed in iodine chamber by dipping the plate in it, till the coloured spot appears.
c. RF value was calculated by formula:

RF value = Distance travelled by solute/ Distance travelled by solvent
= 4.3/4.9
=0.87

Fig.7: *Tridax procumbens* extract

Fig.8:Thin layer chromatography

Paper chromatography

1) Stationery phase: Silica gel

2)Mobile phase:

Toluene : ethyl acetate: formic acid

5 : 4 : 0.2

Paper chromatography Calculations :

RF value was calculated by formula:

$$\text{RF value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

RF value :

= 4.5/5.5
= 0.81

Fig.9: Paper chromatography

RESULT:

- Thin layer chromatoghraphy:

Sr. No.	Spot of sample	Start time	End time	Colour observed	R_F value
1.	*Tridax procumbens*	1:00 pm	1.50 pm	Yellowish green	0.87

- Paper chromatography:

Sr. No.	Spot of sample	Start time	End time	Colour observed	R_F value
1.	*Tridaxprocumbens*	1:00 pm	1.50 pm	Yellowish green	0.81

CONCLUSION

Flavonoids were identified using a variety of methods. The presence of chemicals, primarily flavonoids, in the fractions derived from the ethanolic extract of Tridax procumbens leaves was confirmed by the identification of certain spots using thin layer chromatography and paper chromatography.Flavonoids was found to be present in *Tridax procumbens*.

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