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PHARMACOGNOSTICAL, PHYTOCHEMICAL & ANALGESIC POTENTIAL OF TRIDAX PROCUMBENS PLANT

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ABSTRACT

Tridax procumbens Linn. Commonly known as 'Ghamra' and in English popularly called 'Coat Buttons' because of appearance of flowers which has been extensively used in Ayurvedic system of medicine for various ailments and is dispensed for "Bhringraj" by some of the practitioners of Ayurveda which is well known medicine for liver disorders. The aim of the present study was to evaluate Pharmacognostical, Phytochemical and Analgesic Potential of Tridax procumbens Plant. Establishment of Pharmacognostic profile of the Plant will assist in standardization which can guarantee quality, purity and identification of samples. In the present study we describe Pharmacognostical feature, including macroscopy, microscopy and analytical profile and to investigate the phytochemical and quantitative determination of Phytoconstituent from the whole plant of Tridax procumbens. These observations would be of immense value in the botanical identification and standardization of the drug in crude form. In this study, the hydro alcoholic extract of Tridax procumbens in doses of 200 mg/kg was used. The treated group with extract of Tridax procumbens, hydro- alcoholic extract showed significant reduction in pain at 200mg/kg dose while standard drug showed reduction in pain at dose 5 mg/kg. This study would help distinguish the drug from its adultered species.

***Key Words**: Pharmacognostical, Phytochemical, Analgesic, Tridax procumbens.*

INTRODUCTION

The history of herbal medicine is rather old and dates back to the time when the early man became conscious of his environment. Cultural man is said to have been on earth for some two or three million years and throughout the greater part of the period, he has struggled for his existence as a hunter-gatherer. Thousands of year's experience, by trial and error must have taught him to distinguish between useful and harmful plants with their properties as healing agents dawning on him much later. Since then, medicinal plants have been used in virtually all cultures as a source of medicine. The earliest record of human civilization and culture of China, Egypt, Australia, and Indian valley reveals that the elders and wise men of those times used herbal medicines to treat various diseases. Information regarding these medicinal herbs is available in the old literature, folklore, mythological stories, epic poems, medical treatises and thousands years old manuscripts, palm leaves and copper plates and other records on these cultures which are preserved even today. The excavation of Shanidar cave in Iraq in 1963 revealed the grave of Neanderthal man buried sixty thousand years ago along with many flowers of his time. The plants found in the grave were later identified to having various medicinal properties [1, 2].

One of the earliest records of the use of herbal medicine is that of Chaulmoogra oil from *Hydnocarpus gaertn*, which was known to be effective in the treatment of leprosy. Such a use was recorded in the pharmacopoeia of the Emperor of China between 2730 and 3000 B.C. Similarly, the seeds of the opium poppy (*Papaver somniferum*) and castor seed (*Ricinus communis*) were excavated from some ancient Egyptian tombs, which indicated their use in that 3 part of Africa as far back as 1500 B.C. The records available in "Ebers papyrus" also confirm that medicinal plants were used at that time in Egypt [3]. According to medicinal records, the Materia Medica of Hippocrates, who is now referred to as the father of medicine consisted essentially of herbal recipes, some 400 simple remedies having been compiled and described by him.

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Theophrastus of Athens (370-287 B.C.) was another famous biologist-botanist who produced a number of manuscripts including the famous Historia plantarium. About 500 plants, mostly cultivated, were described in this manuscript [4]. Pliny, the elder (23-79 A.D.), a Roman naturalist and philosopher, described 1000 plants with their medicinal properties, anatomy and horticultural practices in his book, Historia Naturalis. Dioscorides (60 A.D) wrote "De Materia Medica" describing 600 plant species of medicinal value from Mediterranean region. Another manuscript, the Alicia Juliana Codex, was prepared for the daughter of Byzantine Emperor about 512 A.D. from material originally compiled by Dioscorides [2, 4]. In the middle Ages, the writing of Galen (131 A.D.) becomes popular. Galen is considered today to be the most distinguished physician of antiquity after Hippocrates. He treated diseases essentially by the use of herbs. Allopathic as well as homeopathic systems of medicine today are based on the doctrine explained by Galen [3]. The ancient use of plants for healing purposes forms the origin of much of modern medicine. Many conventional drugs originate from plant sources: a century ago, most of the few effective drugs were plant based. Examples include aspirin (from willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from opium poppy). The development of drugs from plants continues, with drug companies engaged in large-scale pharmaceutical screening of herbs [5]. During the last decade, the use of TM (traditional medicine) has expanded globally and has gained popularity. It has not only continued to be used for primary health care of the poor in developing countries, but has also been used in countries where conventional medicine is predominant in the National health care system [6]. It has been confirmed by WHO that herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries [7].

Literature available from all possible scientific sources revealed very little research work on this selected medicinal plant, whereas folklore claim that *Tridax procumbens*, were used in treatment of various diseases and ailments, and they claim for their promising activity but there is no inbuilt scientific proof in support of the utility of this plant or plant products against analgesic activity. So, the present study is investigated to exploring the details of Phytochemical, Pharmacognostical & Analgesic Potential of hydro alcoholic extract from *Tridax procumbens* by using experimental animal models.

MATERIALS AND METHODS

Plant Material:

The plant *Tridax procumbens* belonging to family "Compositae" are available in Himachal Pradesh. For Present work the plant *Tridax procumbens* was collected in the month of April 2015, from Campus of Himachal Institute of Pharmaceutical Education & Research, Nadaun. After collection of plant, it was authenticated by Mr. Vinod Kumar at Rajasthan University. After identification, a voucher specimen was kept at the Department of Botany in Rajasthan University, Jaipur.

Treatment: (Drying):

The whole plant was collected and washed with water. Kept in sunlight for thirty minutes and then dried under shade. Then it was powdered by means of a Grinder.

Physiochemical Parameters:

Foreign Matter:

The 50 gm sample was spread in a thin layer, and the pieces of foreign matter were sorted out by visual inspection. The powder of foreign matter was sifted through a 250 micron sieve. All portions of the foreign matter were pooled and weighed.

Loss on Drying:

10 gm of the drug was weighted in a tarred evaporating dish. It was dried at 105°C for 5 hours and weighed. The drying and weighing was continue at 1 hour interval until difference two successive weighing correspond not more than 0.25% .

Total Ash value:

About 3 gm accurately weighed powdered drug was incinerated in a silica dish at a temperature not exceeding 450^{0}C until free from carbon. It was then cooled and weighed. The % w/w of ash with reference to the air-dried drug was calculated.

Acid insoluble ash value:

To the crucible containing the total ash was added 25 ml of hydrochloric acid. The crucible was then covered with a watch-glass and the mixture was boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot water and this liquid was added in to the crucible. The insoluble matter was collected on an ashless filter-paper and washed with hot water until the filtrate was neutral. The filter-paper contain the insoluble matter was transferred to the original crucible, dried on a hotplate and ignite to constant weight. The residue was allowed to cool in a desiccator for 30 minutes and then weighed.

Water soluble ash value:

Total ash obtained was boiled for 5 minute with 25 ml of water. Insoluble matter was collected in a crucible or an ashless filter paper. Washed with hot water and ignite for 15 minute at temperature not exceeding 450°C. Weight of insoluble matter was substracted from the weight of the ash, the difference in weight was representing the water soluble ash. Percentage of water soluble was calculated with reference to the air dried drug.

Extractive Values:

Alcohol soluble extractive value:

5 gm of the air dried coarsely powdered drug was macerate with 100 ml of alcohol of the specified strength in a closed flask for 24 hour. Shaking frequently during 6 hour and allowed to stand for 18 hour. Filter rapidly and evaporate 25 ml of the filtrate to dryness in tarred flat bottomed shallow dish and dry at 105°C to constant weight and weigh.

Water soluble extractive value:

5 gm of the air dried coarsely powdered drug was macerate with 100 ml of distilled water in a closed flask for 24 hour. Shaking frequently during 6 hour and allowed to stand for 18 hour. Filter rapidly and evaporate 25 ml of the filtrate to dryness in tarred flat bottomed shallow dish and dry at 105°C to constant weight and weigh [8].

Pharmacognostical Parameters:

Macroscopical studies:

Macroscopic studies were carried out by using organoleptic evaluation method. The shape, size, colour, odour, taste, base, texture, margin, apex of leaves and various plant parts of *Tridax procumbens* were observed.

Microscopic studies:

Microscopic studies were carried out by preparing thin sections of leaf and stem. The thin sections were collected in watch glass and bleached with bleaching agent along with little boiling. Thin sections were further washed with water, stained with safranin and mounted in glycerin for observation. Same time quantitative microscopy also carried out with help of plant leaves.

Extraction:

The powdered plant material (40g) was extracted with combination of ethanol and distilled water (50:50) in Soxhlet extractor for 48 hours. After this procedure, extracts was filtered through Whatman No.1 filter paper. This crude sample was then subjected to Phytochemical Investigation and Analgesic action. [9]

Phytochemical Screening:

The following procedures were adapted to tests for the presence of various chemical constituents in extracts.

Tests for Carbohydrates:

Molish's test (general test):

To 2-3 ml aqueous extract, added few drops of α-naphthol solution in alcohol, shaken and added concentrated H_2SO_4 from sides of the test tube was observed for violet ring at the junction of two liquids.

A. For Reducing Sugars:-

- ***Fehling's test:*** 1 ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one minute. Added equal volume of test solution. Heated in boiling water bath for 5-10 min was observed for a yellow, then brick red precipitate.
- ***Benedict's test:*** Equal volume of Benedict's reagent and test solution in test tube were mixed. Heated on boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in test solution.

B. Tests for Monosaccharides:

Barfoed's test: Equal volume of Barfoed's reagent and test solution were added. Heated for 1-2 min, on boiling water bath and cooled. Observed for red precipitate.

C. Tests for Hexose sugars:

Cobalt-chloride test: 3 ml of test solution was mixed with 2ml cobalt chloride, boiled and cooled. Added $Fecl_3$ drops on NaOH solution. Solution observed for greenish blue (glucose), purplish (fructose) or upper layer greenish blue and lower layer purplish (mixture of glucose and fructose).

D. Tests for Non-reducing sugars:

- Test solution does not give response to Fehling's and Benedict's test.
- Tannic acid test for starch: with 20% tannic acid, test solution was observed for precipitate.

Tests for Proteins:

- ***Biuret test*** *(general test):* To 3 ml t.s added 4% NaOH and few drops of 1% c_4so_4 solution observed for violet or pink colour.
- ***Million's test*** *(for proteins):* Mixed 3 ml t.s. with 5 ml Million's reagent, white precipitate. Precipitate warmed turns brick red or precipitate dissolves giving red colour was observed.
- ***Xanthoprotein test*** *(for protein containing tyrosine or tryptophan):* Mixed 3ml t.s. with 1 ml concentrated H_2SO_4 observed for white precipitate.
- ***Test for protein containing sulphur:*** Mixed 5 ml t.s. with 2 ml 40% NaOH and 2 drops 10% lead acetate solution. Solution was boiled, it turned black or brownish due to Pbs formation was observed.

Tests for Amino Acids:

- **Ninhydrin test (general test)**: 3 ml t.s. and 3 drops 5% Ninhydrin solution were heated in boiling water bath for 10 min. Observed for purple or bluish colour.
- **Test for Tyrosine**: Heated 3 ml t.s. and 3 drops Million's reagent. Solution observed for dark red colour.
- **Test for Tryptophan**: To 3 ml t.s. added few drops glycoxalic acid and concentrated H_2SO_4 observed for reddish violet ring at junction of the two layers.

Tests for Glycosides:

A. Tests for Cardiac Glycosides:

- ***Baljet's test:*** A test solution observed for yellow to orange colour with sodium picrate.
- ***Legal's test (for cardenoloids):*** To aqueous or alcoholic test solution, added 1 ml pyridine and 1 ml sodium nitroprusside observed for pink to red colour.
- ***Test for deoxysugars (Kellar-killani test):*** To 2 ml extract added glacial acetic acid, one drop of 5% $Fecl_3$ and concentrated H_2SO_4 observed for reddish brown colour at junction of the two liquid and upper layers bluish green.
- ***Libermann's test (for Bufadenolids):*** Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H_2SO_4 observed for blue colour.

B. Test for Anthraquinone Glycoside:

- ***Borntrager's test:*** Boil powder drug with 5ml of 10% sulfuric acid for two minutes. Filter while hot. Cool the filtrate and shake gently with equal volume of benzene. Separated benzene layer is treated with half of its volume of solution ammonia (10%). Allow to separate. Ammonical layer acquires rose pink colour due to the presence of anthraquinones.
- ***Modified Borntrager's test:*** C-glycosides of anthraquinones require more drastic condition for hydrolysis. Hydrolysis of drug is carried out with 5ml of dilute HCl and 5ml of 5% solution of ferric chloride. Carry out procedure as described under Bontrager's test.

C. Tests for Saponin Glycosides:

- ***Foam test:*** The drug extract or dry powder was shacked vigorously with water. Persistent foam was observed.
- ***Hemolytic test:*** Added test solution to one drop of blood placed on glass slide. Hemolytic zone whether appeared was observed.

D. Tests for Coumarin Glycosides:

Test solution when made alkaline, observed for blue or green fluorescence.

Tests for Flavonoids:

- ***Shinoda test:*** To dried powder or extract, added 5 ml 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings. Pink colour was observed.
- ***To small*** quantity of residue, added lead acetate solution observed for yellow coloured precipitate.
- ***Addition of increasing amount*** of sodium hydroxide to the residue whether showed yellow coloration, which was decolorized after addition of acid was observed.
- ***Ferric chloride test:*** Test solution, added few drops of ferric chloride solution observed for intense green colour.

Tests for Alkaloids:

- ***Dragendroff's test:*** To 2-3 ml filtrate added few drops Dragendroff's reagent observed for orange brown precipitate.
- ***Mayer's test:*** 2-3 ml filtrate with few drops Mayer's reagent observed for precipitate.
- ***Hager's test:*** 2-3 ml filtrate with Hagers reagent observed for yellow precipitate.
- ***Wagner's test:*** 2-3 ml filtrate with few drops of Wagner's reagent observed reddish brown precipitate.

Tests for Tannins and Phenolic compounds:

To 2-3 ml test solution, added few drops of whether showed following was observed:

- 5% $Fecl_3$ solution: deep blue-black coloured.
- Lead acetate solution: white precipitate.
- Gelatin solution: white precipitate.
- Bromine water: discoloration of bromine water.
- Acetic acid solution: red colour solution.
- Potassium dichromate: red precipitate.
- Dilute Iodine solution: transient red colour.
- Dilute HNO_3: reddish to yellow colour [10].

Pharmacological Screening:

Analgesic Activity:

Preparation of test extract:

Standard drug used for treatment Diclofenac Sodium (5 mg/kg), Hydro- alcoholic extract (200 mg/kg) was prepared and given intra peritoneally. The dose of 200 mg/kg of extract and standard were made by dissolving appropriate quantity of extracts in Normal Saline solution.

Grouping & Treatment of Experimental Models:

Male Albino rats weighing 150-200 gms were divided in to three groups each consisting of six animals (n=6).

Group I : Served as control,
Group II : Received hydro - alcoholic extract, 200mg/kg,
Group III : Served as reference standard
(Diclofenac Sodium 5 mg/kg)

Experimental Procedure:

The Analgesic activity was tested by tail flick method in Male Wistar Albino rats. After overnight fasting healthy albino rats were divided three groups with six animals in each group. The tail flick latencies of the animals were assessed by Analgesiometer, basal reaction time was taken by placing the tip (last 2.5 cm) of the tail on the radiant heat source. Tail withdrawl from the heat (flicking response) was taken as the end point. The cut-off reaction time was fixed at 10 sec to avoid tissue damage.

The mean reaction time was recorded before treatment & an interval of 60, 90, 120, 150 and 180 min. after administration of vehicle or drugs by i. p. route. Diclofenac Sodium used as standard drug for comparing the analgesic action of plant extract [11].

RESULTS

Physico-Chemical Parameter:

The Physicochemical parameters were investigated and reported in table no. 1. The above studies enable the identification of the plant material for future investigation and form an important aspect of drug studies.

Table No. 1: Physiochemical Parameters of *Tridax procumbens*

S. No.	Parameters	Values Obtained
1	Foreign Matter	1 %
2	Moisture Content	2.50%
3	Total Ash	10.62%
4	Acid Insoluble Ash Value	2.06%
5	Water Soluble Ash Value	1.54%
6	Alcohol Soluble Extractive Value	6.12%
7	Water Soluble Extractive Value	27.16%

Pharmacognostical Evaluation:

Macroscopy:

Stems decumbent, producing roots at the nodes, up to 50 cm tall. Stems clothed in pale hairs. Leaf blades 30-60 x 15-35 mm, clothed in hairs, lateral veins 2-3 on each side of the midrib. Petioles hairy, 5-10 mm long. Flowers produced in heads about 10 x 10-12 mm. Peduncles hairy, 11-20 cm long. Heads surrounded by bracts, the outer bracts hairy, each bract about 7 x 4 mm, inner bractsglabrous, 7-8 mm long. Calyx (pappus) consists of barbed or fimbriate hairs 10-12 mm long. Corolla on the ray florets ligular, 9-10 x 4 mm, apex 3-lobed. Corolla of the disk florets tubular, about 5 mm long, corolla lobes about 0.5 mm long. Stamens fused to form a tube. Ovary 2-2.2 x 1 mm, densely clothed in long pale brown or golden hairs. Fruit (achene) 1.6-2 mm long; pappus of slender, plumose bristles 5- 6 mm long, with fine spreading hairs.

Quantitative Investigation:

Quantitative microscopy includes stomatal number, stomatal index, palisade ratio, vein-islet number and vein termination number. The value obtained for leaf constant is tabulated in Table 2.

Table No. 2 : Quantitative Investigation of *Tridax procumbens*

Sample Identity	Type of Stomata	Stomatal Index		Vein Islet No. per mm²		Vein Termination No. per mm²	
		Upper	Lower	Upper	Lower	Upper	Lower
Leaves	Anomocytic	28.12	34.21	30.18	18.16	30.36	17.16

Microscopy:

Transverse section shows single layered palisade cells just below the epidermis followed by 5-7 celled parenchymatous cells. Simple trichomes with multicelled. Presence of vascular bundle with surrounding of dark parenchymatous cell is main character. T.S. passing through the mid rib region shows slight depression on ventral side and slightly protuberated on dorsal size.

Phytochemical Investigation:

The extracts obtained were subjected to various phytochemical tests, to identify the active constituents, which show in table no. 3. From this analysis, extract show presence of Carbohydrate, Proteins, Glycosides, Flavonoids, Tannins and Saponins are determined.

Table No. 3 : Phytochemical Investigation of *Tridax procumbens*

S. No.	Phytoconstituent	Status
1	Carbohydrates	Positive
2	Proteins	Positive
3	Amino Acids	Negative
4	Glycosides	Positive
5	Alkaloids	Negative
6	Tannins	Positive
7	Flavonoids	Positive

Pharmacological Investigation:

Analgesic Activity:

The Analgesic activity was tested by tail flick method in Albino rats using Analgesiometer. The standard drug was served as Diclofenac Sodium. The hydro- alcoholic extract (200 mg/kg) was selected for the analgesic study. The results were interpreted by measuring the decrease in pain after given test extract and standard drug.

Decrease in is observed at 60, 90, 120, 150 and 180 min. after the treatment with the extract and standard drug tabulated in Table no.4.

Table No. 4: Analgesic Activity of Hydro – Alcoholic extract of *Tridax procumbens*

Treatment ↓ Minute →	Dose (mg/kg)	Analgesia Time (Minutes)				
		60	90	120	150	180
Normal Saline	0.2 ml	0.62 ± 0.17	0.386 ± 0.3	0.33 ± 0.29	0.50 ± 0.12	0.367 ± 0.27
Tridax procumbens	200	0.91 ± 0.16	1.30 ± 0.80	2.10 ± 0.10	2.13 ± 0.78	3.90 ± 0.21
Diclofenac Sodium	5	0.95 ± 0.15	2.21 ± 0.16	2.21 ± 0.12	3.28 ± 0.29	3.74 ± 0.21

The treated group with extract of *Tridax procumbens*, hydro- alcoholic extract showed significant reduction in pain at 200mg/kg dose while standard drug showed reduction in pain at dose 5 mg/kg. The results were found to be significant ($p<0.05$) in comparison to control.

DISCUSSION AND CONCLUSION

The plant *Tridax procumbens* has been examined to gain an insight of its Pharmacognostical and Pharmacological behavior. In the present study, whole plant extract of *Tridax procumbens* was subjected to analgesic activity. The authenticated plant material was subjected to physiochemical standardization and phytochemical investigation.

- All the Physicochemical parameters were investigated for *Tridax procumbens*. The above studies enable the identification of the plant material for future detailed investigation.
- The Pharmacognostical investigation revealed that the plant contain Simple Trichome, vascular bundle surrounded by some parenchymatous cells filled with dark content.
- The Phytochemical investigation revealed that the plant contain Glycosides, Flavonoids, Saponins or tannins which may be responsible for activity.
- The Analgesic activity of *Tridax procumbens* extract was studied for central analgesic activity. Tail flick method is used for detecting analgesic activity. The analgesic activity of hydro - alcoholic extract against acute inflammatory pain was moderate as compared to potent inhibitory activity of Diclofenac Sodium. In the present study, hydro - alcoholic extract (200 mg/kg) significantly increased the reaction time in tail flick method, suggesting its central analgesic activity. Hydro - alcoholic extract showed significant reduction in pain at 200mg/kg dose while standard drug showed reduction in pain at dose 5 mg/kg. The results were found to be significant ($p<0.05$) in comparison to control.

Indian herbal Drugs used at worldwide level because of their acceptability and less side effects in developed countries. So it is necessary to identify and standardize crude drugs for their Purity. Based on the results obtained from the present study, it can be inferred that test extracts had effective peripheral analgesic. The results of the present study justify that the use of *Tridax procumbens* plant in pain by Folklore Civilization. More study need to be done to establish the mechanism by which the plant exerts its pharmacological effect.

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