INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

PHRMACOGNOSTICAL AND PHARMACOLOGICAL SCREENING OF *TRIDAX PROCUMBENS* L.

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Article Received on: 12/05/11 Revised on: 23/06/11 Approved for publication: 09/07/11

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ABSTRACT
Pharmacognostical parameters for the leaves of *Tridax procumbens* L. (Family- Asteraceae) were studied with the aim of drawing the pharmacopoeial standards for this species. Macroscopical and microscopical characters, physico-chemical constants, extractive values of dry powder and its reaction after treatment with chemical reagents were studied. However, its functional properties and the underlying mechanism of action have not been clearly defined the present study was undertaken to evaluate anthelmintic activity of ethanol and aqueous extract of leaves of *Tridax procumbens* L. against *Pheretima posthuma*. Various concentrations (50- 150 mg/ml) of ethanol & aqueous extracts were evaluated in the bioassay involving determination of time of paralysis (P) and time of death (D) of the worms. Piperazine citrate was used as standard anthelmintic drug and distilled water was used as control. The results of present study indicated that the ethanol extract significantly exhibited paralysis (P<0.01) in worms in doses (50- 150 mg/ml). Further studies are in process to isolate the active principle/s responsible for the activity
Keywords: Pharmacognostic, Phytochemical, Anthelmintic activity, *Tridax procumbens* L, Asteraceae.

INTRODUCTION

Tridax procumbens L. is commonly known as 'Ghamra' in Hindi and in English popularly called 'Coat button' because of appearance of flowers[1].A hispid, procumbent herb, with woody base, some time rooting at nodes, up to 60 cm. high[2,4], found as a weed in cultivated and other disturbed habitats throughout India to an altitude of 2,400m[3]. Leaves cooked as a vegetable[2]; they are also eaten by cattle[4]. Aerial parts of *Tridax procumbens* L. (TP) reported Immunomodulatory effects[4]. Aerial parts showed Hepatoprotective activity of TP against D-galactosamine/ lipopolysaccharide-induced hepatitis in rats[5]. Leaves are reported hemostatic activity[6], effect on Blood pressure and Heart Rate in rats[7] and Anti-diabetic activity[8]. The occurrence of β-sitosterol-3-O-β-D-xylopyranoside[9], lipid constituents[10], saturated and unsaturated fatty acid from TP[11]. The whole plant and seed being used to treat a variety of ailments the leaves are cooked and eaten as a vegetable. But no Pharmacognostical work has been done so far. Therefore, an attempt has been made to study the Pharmacognostical parameters and anthelmintic activity on the leaves of TP.

Helminthes plays a crucial role in the small ruminant production leading to enormous economic losses particularly in areas where extensive grazing is practiced[12]. It is also being recognized as cause of many acute as well as chronic ill health among the various human beings as well as cattle's. Development of resistance to most of the commercially available anthelmintics became a severe problem worldwide. Moreover, these drugs are unaffordable, inaccessible or inadequately available to the resource-poor farmers of the developing countries[13]. These factors paved the way for the herbal formulation as alternative anthelmintics. In the current study, we have attempted to investigate the traditional polyherbal formulation for their anthelmintic activity.

MATERIALS AND METHODS

Plant Collection and authentication

The plant material was collected from the National Botanical Research Institute Garden, Lucknow, in the month of June. The plant was identified and authenticated by Chemotaxonomist, NBRI Lucknow, and the Accession No. is 94484.

The fresh leaves were dried under the shade and powdered using mechanical grinder. Then the powder was passed through sieve no. 40 to get the desired coarseness. Powdered material was preserved in an air tight container for further use.

Chemicals

All chemicals and reagents used for testing were of analytical grade purchased from SD Fine Chemicals, Mumbai.

Macroscopical characters

It is dark green, fine, odorless powder with slight bitter taste. The powder microscopy reveals the presence of different types of (Glandular and Non Glandular) Trichomes, trichome base, fibres, stone cells, laticifers with adjacent parenchyma. Spiral thickenings vascular bundles.

The macroscopical characters (size, shape colour, odour, texture, margin, base, apex and petiole) of the leaves were observed[3, 4]. Then, anatomical study, powder was identified with routine reagents to study the lignified cells, trichomes, stomata, fibres etc.

Microscopical character

The leaves, seeds and root were selected for the microscopical study. Microscopic sections were cut by using microtome and free hand sectioning. Numerous temporary and permanent mounts of the microscopical sections of the leaves specimen were made and examined.[14]

Physico-chemical parameters

The ash values, extractive values with various reagents and were determined as per the Indian Pharmacopoeia[15].

Extractive values

Extractive values were performed with various solvents like hexane, ethanol and water was performed as per standard procedure[15].

Phytoconstants

Measurement of vein islet number, vein termination number, stomatal number, stomatal index and length of trichome were determined[16].

Preliminary Phytochemical screening

Phytochemical screening is done for analyzing secondary metabolites, which are responsible for curing ailment. Preliminary Phytochemical tests of the powder were performed using specific reagents through standard procedures[17,18].

Pharmacological screening

Indian earthworms *Pheretima posthuma* (Annelida) were collected from the water logged areas of soil worms were obtained from freshly slaughtered fowls (Gallus gallus). Worm types were identified at the Agriculture Research Station, Aland road, Gulbarga.

Preparation of Standard Solution:

Piperazine citrate (15mg/ml) was used prepared by using 0.2% v/v of Tween 20 as a suspending agent. Piperazine citrate was used as standard.

Preparation of Extract:

The different concentration (50-150 mg/ml) suspension of both extract of leaves of TP were prepared by using 0.2% v/v of Tween 20 as a suspending agent and final volume was made to 10 ml for respective concentration of leaves of TP.

Eight groups of approximately equal size worms consisting of six earthworms individually in each group were released into 10 ml of desired concentration of Std. drug and extracts.

Experimental Design

Eight groups of approximately equal size worms consisting of six earthworms individually in each group were released into 10 ml of desired concentration of drug. The anthelmintic assay was carried out as per the method [19] with minor modification.[20,21]. The animals were divided into eight group containing six earthworms each different concentration of extracts and standard drug solution were poured in different Petri dishes. Observations were made for the time taken for paralysis (Paralysis was said to occur when worm did not revive in normal saline) and death (Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50^0C), followed with their body colors fading away) [22,23].For evaluation of anthelmintic activity of leaves of TP , group I was control , group II received 15mg/ml Piperazine citrate, group III, IV & V received 50,100, 150 mg/ml ethanolic extract respectively. While group VI, VII & VIII received 50,100, 150 mg/ml aqueous extract of leaves of TP. All the results were expressed as Mean ±S.D. of six animals in each group.

Statistical analysis

All results are expressed as mean ±standard error. The data was analyzed using two ways of analysis of variance (ANOVA). The statistical significance of the difference of the means was evaluated by Dunnet's test.

RESULTS AND DISCUSSION

Macroscopical characters

Morphological study was carried out for organoleptic evaluation. In that study color, structure, shape and size were visually observed. (**Table no 1).**

Microscopical character

T.S. passing through the laminar region shows single layered palisade cells just below the appear epidermis followed by 5-7 celled mesophyll parenchyma mostly devoid of inter cellular spaces.

Petiole

Kidney shaped towards the distal end and crescent shaped towards the laminal side. Single layered epidermis covered with cuticle and interrupted by simple, multicellular, 3-5 celled trichomes. Hypodermis 1-2 celled collenchymatous. Ground tissue

parenchymatous; vascular bundles 5, the size of the vascular bundles various from centre to margin i.e. Large to small. These are centripetal i.e. xylem surrounded by the phloem.

Leaf

T.S. leaf is dorsiventral, epidermis single layered on both the surfaces and covered with thick cuticle. T.S. passing through the mid rib region shows slight depression on ventral side and slightly protuberated on dorsal size. Trichomes are simple, multicelled (3-6 celled) and more in number on dorsal side. The basal cells of the Trichome are swollen and Trichome looks like claw. Meristeel consists of single centrally located collateral vascular bundle surrounded by some parenchymatous cells filled with dark content.

T.S. passing through the laminar region shows single layered palisade cells just below the appear epidermis followed by 5-7 celled mesophyll parenchyma mostly devoid of inter cellular spaces.

Physicochemical parameters

Ash value

The results revealed that the seeds showed highest total ash and acid insoluble ash value. The root showed more water soluble ash value as compare to other parts of TP**. (Table 2)**

Extractive value:

The results revealed the highest extractive value for leaves was from water. However, the ethanol soluble extractive value was greater than hexane **(Table 3)**

Leaf constants

Quantitative microscopical study also give valuable information regarding specific leaf constants such as vein islet upper and lower epidermis, vein termination number upper and lower epidermis, stomata index upper and lower epidermis. Trichome numbers, upper and lower epidermis Loss on drying as shown in (**Table 4)**.

Preliminary Phytochemical screening

Preliminary Phytochemical screening revealed the presence glycoside, flavonoids, Tannins, mucilage and carbohydrate. [13, 14] **(Table 5)**

Anthelmintic activity:

The extracts of TP produced a significant anthelmintic activity in dose dependent manner. The ethanol extract caused paralysis of 32.33±4.50min and time of death of 83.33±3.05 min., while aqueous extract revealed paralysis of 48.66±4.04 min and time of death of 109.66±3.05min against the earthworm *Pheretima posthuma*. The activity of both ethanolic and aqueous extract was comparable with that of standard drugs.**(Table 6)**

DISCUSSION

Leaves were green**,** characteristic odours with slight bitter taste. Leaves are of size 3-7 cm in length, lanceolate in shape, acute – apex, wedge-shaped base, irregularly toothed margin, and short petiole as shown in **Table 1**. The physical constants such as total ash value (11.88%), acid insoluble ash (3.05%), water soluble ash (2.14%), sulphated ash (20.11) as shown in **Table 2** and extractive values are specific identification as shown in **Table 3**. The soluble extractive values with different solvents such as Hexane, ethanol and water were (8.90%, 07.17% and 28.16%) respectively, which indicates the nature of constituents present. Quantitative microscopical study also give valuable information regarding specific leaf constants such as vein islet upper and lower epidermis(32.33/mm^2 and 20.66/mm^2),vein termination number upper and lower epidermis (32.66/mm^2 and 19.66/mm^2), stomatal index upper and lower epidermis (30.12 and 36.31). Trichome number upper and lower epidermis (9.66 and 21.33). Loss on drying (13.00%) as shown in **Table 4**. The behavior of leaf powder upon treatment with different chemical reagents was also observed and reported in **Table 5**. Preliminary Phytochemical screening revealed the presence glycoside, flavonoids, Tannins, mucilage and carbohydrate.

The results of the anthelmintic study demonstrated that, the ethanolic extract of TP shows potent anthelmintic activity with varying magnitudes. But the extract of TP showed highest activity, which is almost equal in effectiveness to standard Piperazine citrate. The difference in the time taken for induction of paralysis in both Piperazine citrate and Trikatu churna was insignificant or almost same. However, significant difference was observed when compared the induction of paralysis time of Piperazine with aqueous extracts. The mode of action for the Piperazine is generally by paralyzing parasites, which allows the host body to easily remove or expel the invading organism **(Table 6).**

REFERENCES

1. Vyas PS, Tiwari U, Rastogi B, Singh P. Immunomodulatory effects of aqueous extract of *Tridax procumbens* in experimental animals. Journal of Ethnopharmacology 2004; 113-119.
2. Asima C. the Treatise of Indian medicinal plants. (Satyesh Chandra Prakashan, CSIR, New Delhi).2001; Vol. 5:180
3. Parrota JA. Healing plants of peninsular India. (CAB International publishing) 2001;157-158
4. The Wealth of India: Raw materials, (Publication and Information Directorate, CSIR, New Delhi). 1988; 87-88.
5. Thiruvengadam D, Vilwanathan R, Shivashangari SK. Hepatoprotective activity of *Tridax procumbens* against D-galactosamine/lipopolysaccharide-induced hepatitis in rats. Journal of Ethnopharmacology 2005; 55-60.

6. Dhake AS, Kale AM, Shahi RS, Shamkuwar PB, Somani VG. Hemostatic activity of leaves of *Tridax procumbens* L. International J of Green Pharmacy 2008; 54.
7. Salahdeen MH, Yemitan KO, Alada ARA. Effect of aqueous leaf extract of *Tridax procumbens* on Blood pressure and Heart rate in rats. African J of Biomedical Research 2004; 27-29.
8. Bhagwat DA, killedar SG, Adnaik RS. Anti-diabetic activity of leaf extract of *Tridax procumbens.* International J of Green Pharmacy 2008; 2(2): 126-128.
9. Albert S, Saxena VK. β-sitosterol-3-O-β-D-xylopyranoside from the flower of *Tridax procumbens*. J Chem Sci.2005; Vol.-3: 263-266.
10. Gupta MM and Verma RK. "Lipid constituents of *Tridax procumbens*" Phytochemistry1988; 27(2): 459-463.
11. Gabhe SY, Gadre AP. saturated and unsaturated fatty acids from Tridax procumbens" Indian J Pharm Sci1988; 168.
12. Waller PJ. Sustainable helminth control of ruminants in developing countries. Vet Parasitol 1997; 71: 195-207.
13. Fajmi AK, Taiewo AA. Herbal remedies in animal parasitic diseases in Nigeria: A review. Afr J Biotech 2005; 4:303-7.
14. Wahi AK and Geetha M. Pharmacognostical studies on leaves of Barleria prionitis *Linn.* Indian Journal of Natural Products 2002; 16: 16-19.
15. Anonymous, the Indian Pharmacopoeia (Govt. of India publication, New Delhi), 1966:p. 947-950.
16. Wallis TE, Text Book of Pharmacognosy (CBS publishers and Distributors, Delhi) 1989. p. 356-549
17. Khandelwal KR, Pawar AP, Kokate CK and Gokhale SB. Practical Pharmacognosy. Pune: Nirali Prakashan; 2001. p. 153.
18. Kokate CK, Purohit A, Gokhale S. Practical Pharmacognosy. 10th ed. New Delhi, India: Vallabh prakashan; 1994. p. 112-20.
19. Ajayeoba EO, Oncha SA, Olarenwaju OT. In vivo anthelmintic property of Buehholria cariacen & Gyandropis gyancha ext. Pharm Biol 2001;39: 127-210.
20. Guha P. Betel leaf: The neglected green gold of India. J Hum Ecol 2006; 19 (2).
21. Chopra RN, Nayer SC, Chopra IC. Glossary of Indian medicinal plants. CSIR, NewDelhi, 1956; p.160.
22. Kelly JD and Hall CA. resistance of animal helminthes to antihelmintics. Adv Pharm Ther 1979; 16: p. S9-S12.
23. Dash OK, Suresh P, Kar DM, Ganpaty S and Panda SP. Evaluation of Evolvulus alsinoids Linn,for anthelmintics and antimicrobial activities, J N at SRem 2002: 182-185.
24. Wallis TE, Textbook of Pharmacognosy (CBS publishers and Distributors, Delhi). 1985;104-105
25. Evans WC; Treas and Evans "Pharmacognosy" (Fourteenth Edi, WB Saunders company Ltd). 2002. p. 3-4
26. Johansen DA, Plant Micro technique (McGraw Hill Book Co., New York); 1940. p.182-197.

Table 1: Macroscopical characters of leaves of TP

Parts	Observation
Part	Leaves
Arrangement	Opposite
Size	3-7 cm long, 1- 4 cm wide
Shape	Lanceolate to ovate
Colour	Green
Odour	Characteristic
Taste	Acrid
Appearance	Rough & Scabrous
Margin	Irregularly toothed
Apex	Acute
Base	wedge- shaped
Petiole	Short
Texture	Short
Fracture	Easy

Table 2: Determination of Ash Values of leaves of TP

Sr. No.	Ash type	Value % (w/w)
1.	Total ash	11.88
2.	Acid insoluble ash	3.05
3.	Water soluble ash	2.41
4.	Sulphated ash	20.11

Table 3: Determination of Extractive Values of leaves of TP

Sr. No.	Solvent	Value % (w/w)
1.	Hexane	08.90
2.	Ethanol	07.17
3.	Water	28.16

Table 4: Determination of Leaf constants of leaves of TP

Leaf constants	Report
Vein islet number(upper epidermis)	32.33/mm^2
Vein islet number (lower epidermis)	20.66/mm^2
Vein termination number (upper epidermis)	32.66/mm^2
Vein termination number(lower epidermis)	19.66/mm^2
Trichome number (upper epidermis)	9.66/mm^2
Trichome number (lower epidermis)	21.33/mm
Stomata index (upper epidermis)	30.12
Stomata index (lower epidermis)	36.31
Palisade ratio	3.62

Table 5: Preliminary phytochemical screening of leaves of TP

Sr. No.	Chemical tests	
1.	**Alkaloids**	
	Dragendroff's test	-
	Mayer's test	-
	Hager's test	-
	Wagner's test	-
2.	**Glycoside**	
	Anthraquinone glycosides	+
	Cynogenic glycoside	-
	Cardiac glycoside	-
3.	**Flavonoids**	+
4.	**Coumarin**	-
5.	**Tannins**	+
6.	**Saponins**	-
7.	**Mucilage**	+
8.	**Carbohydrate**	+

Where, + - Present; - - Absent

Table 6: Anthelmintic activity of aqueous and ethanolic extract of leaves of TP

Treatment	Group	Concentration (mg/ml)	Time of paralysis (min) (Mean±S.D.)	Time of death (min) (Mean±S.D.)
Control	I	-	-	-
Piperazine citrate(Std.)	II	15	21.66± 6.18**	72.33±2.054**
Ethanolic Extract of TP	III	50	87.00±3.0	191.66±2.51
	IV	100	42.33±3.21*	146.00± 2.0*
	V	150	32.33±4.50**	83.33±3.05**
Aqueous extract of TP	VI	50	97.33±1.15	215.00±3.60
	VII	100	72.00±3.0*	152.00±4.0*
	VIII	150	48.66±4.04**	109.66±3.05**

Values are expressed as Mean±Sem, by Anova Dunnets't' test, n=6 in each group, *P<0.05, **P<0.01.

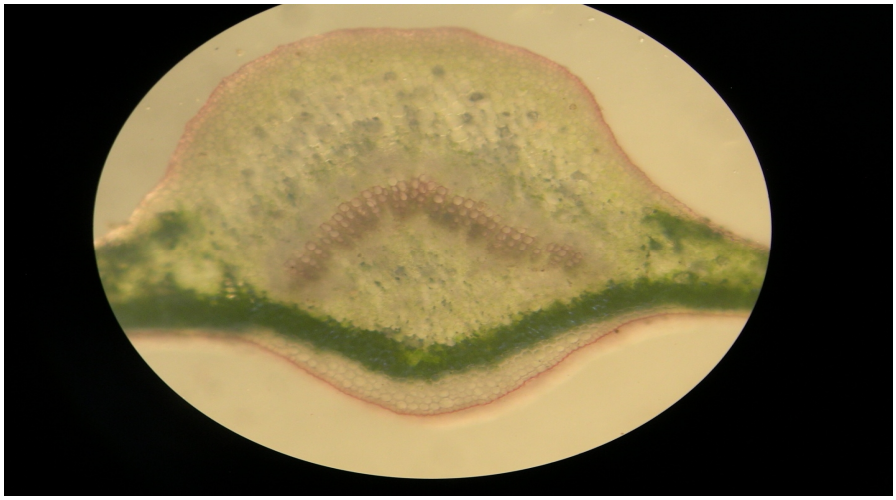

Fig. 1: T.S of Leaf of of of TP.

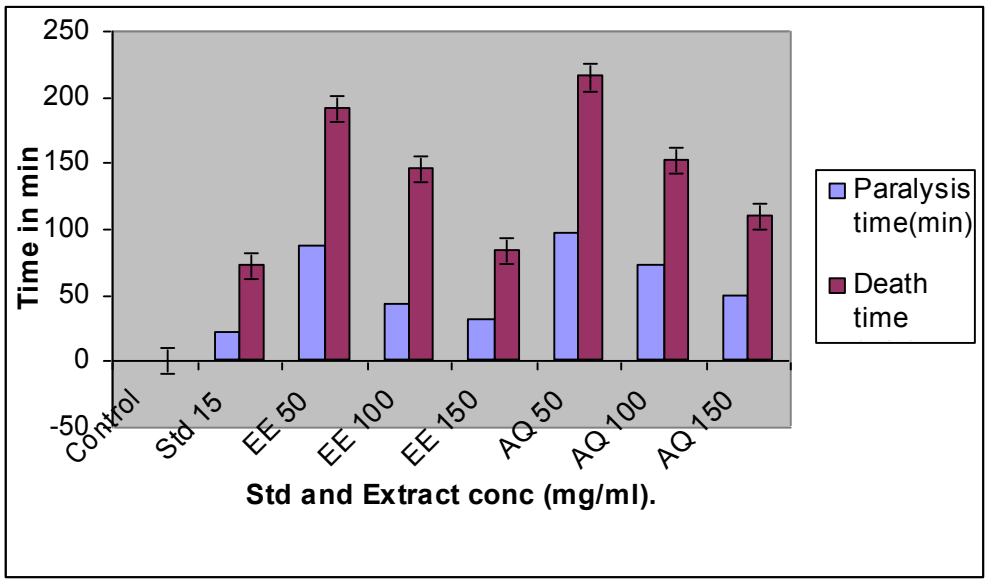

Fig.2: Anthelmintic activity of leaves extract of TP

Source of support: Nil, Conflict of interest: None Declared