

Antihelmintic Activity Of Ethanolic Extract Of Tridax Procumbens Linn

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ABSTRACT

The ethanolic extract of Tridax Procumbens stem found to be statistically significant at the ethanolic extract possessed the crude extract of Tridax Procumbens Linn in water, methanol, ethanol, ethyl acetate, chloroform, were evaluated on adult Indian earthworms for anthelmintic activity.

The stems extract of Tridax Procumbens exhibited a dose dependent inhibition of spontaneous motility (paralysis) and evoked responses to pin-prick. The effects were comparable with that of albendazole wormicidal (worm killing) activity and thus, may be useful as an anthelmintic.

KEYWORDS:-Tridax Procumbens Linn, anthelmintic activity, wormicidal activity.

INTRODUCTION

Helminthiasis: Helminthiasis is a medical condition characterized by the infestation of parasitic worms in the human body. These worms can belong to various classes, such as nematodes (roundworms), cestodes (tapeworms), or trematodes (flukes).Helminth infections are among the commonest infections in man, affecting a large proportion of the world's population.



Fig: Parasitic Hook Worm causing Helminths Infection

In developing countries they pose a major threat to public health and contribute to the prevalence of malnutrition (lack of nutrition), anemia (lack of RBC), eosinophilia (presence of too many group of WBC), and pneumonia(lung infection). **Anthelmintics:** Anthelmintics are drugs that either kill or expel infesting helminths and the gastrointestinal tract is the place of residence of many helminths, although some live in tissues, or their larvae (immature form) migrate into tissues. They harm the host by depriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins. Helminthiasis is rarely fatal, but is a major cause of morbidity.[1]

Helminths parasite infections are global problems with serious social and economic repercussions in the Third World countries. The diseases affect the health status of a large fraction of the human populations well as animals. Some type of dangerous helminths infections like filariasis (mosquito-borneparasitic infection) has only a few therapeutic modalities at present. The continuous and long-term reliance on a small range of compounds has led to the development of drug resistance in many helminthic strains[2]. In addition, after treatment with albendazole ormebendazole, several side effects have been reported in hosts such as gastrointestinal symptoms, nervous system symptoms and allergic phenomena. Some anthelmintic drugs, such as praziquantel and albendazole, are contraindicated for certain groups of patients like pregnant and lactating woman. These drugs have also to be used with caution in hepatitis patients and in children below 2 years of age [3].keeping this in view, the present communication deals with the evaluation of the anthelmintic activity of roots of Tridax procumben.

Tridax Procumbens Linn is a weed found throughout India. It is concluded that the chloroform extract of Tridax Procumbens linn shows potent anthelmintic activity compare to standard anthelmintic drug. A hispid, procumbent herb with woody base sometime rooting at the node, up to 60 cm high. Leaves are ovate-lanceolate 2to 7 cm and lamina pinnatisect, sometimes three lobed; flowers in small, long peduncled heads; achene's 1.5 - 2.5mm long x 0.5 - 1 mm in diameter and densely ascending pubescent; persistent; bristles of disc achenes alternately longer and shorter, 3.5 - 6 mm in length[4] as shown in the below picture.



Fig: Tridax Procumbens Linn

The plant is reported for its pharmacological actions like is commonly used in Indian traditional medicine as anticoagulant, antifungal and insect repellent, in bronchial catarrh, diarrhea and dysentery [5, 6]. Moreover it possesses wound healing activity and promotes hair growth [7]. The leaves are reported to be employed in bronchial catarrh, dysentery or diarrhea and for restoring hair. The leaf gel possesses antiseptic, insecticidal and parasitical properties. It is used to check hemorrhage from the cuts, bruises and wounds. An aqueous extract of plant produces reflex tachycardia (B.P. decreases and heart beat increases) and showed a transient (dysfunction) hypotensive effect on the normal blood pressure. It is employed as an indigenous medicine for a variety of ailments including jaundice. The plant also has hepatoprotective activity and it is used in Ayurveda in various liver disorders [8].

Other Studies:



Fig. Pharmacological action

Although Tridax procumbens L. (Asteraceae) was reported as a weed to invade in many crops, it has been long employed as a traditional drink to cure treat bronchial catarrh, diarrhea, dysentery [9], and liver diseases [10,11] in many countries in Africa, South and Southeast Asia. Many bioactive compounds, such as procumbetin [9], 8,3'-dihydroxy-3,7,4'-trimethoxy-6-O- β -d-glucopyranosyl flavone, 6,8,3'-trihydroxy-3,7,4'-trimethoxyflavone; puerarin [12], centaurein, and centaureidin, have been successfully isolated from this plant [13]. Lipid constituents of this plant, including methyl 14-oxooctadecanoate, methyl 14- oxoonacosanoate, 30-methyl-28-oxodotriacont-29-en-l-oic acid, β -amyrone, β -amyrin; lupeol, and fucosterol, have been identified [14]. Furthermore, phenolic acids, including benzoic, vanilic, benzeneacetic acids, and guiacol, from this plant have been determined [15]

T. procumbens possesses a wide spectrum of biological activities. The ethyl acetate extract of this plant showed strong allopathic and larvicidal activities [15, 16]. In pharmaceutical activities, methanol and ethanol extracts exhibited anti-hyperglycemic [17], anti-fungal [18], anti-leshmanial [19], and hepatoprotective activities [20], while ethyl acetate extract exerted

Anti-inflammatory, anti-cyclooxygenase, and antioxidant activities [13]. The acetone extract of this herb obtained anticoagulant, anti-hepertic, antibacterial activities [21]. However, information on the anti-hyperuricemia property of this plant has not been yet documented.

Hyperuricemia is an abnormality high level of uric acid in the blood. Normal uric acid levels are 2.4–6.0 mg/dL for female and 3.4–7.0 mg/dL for male [22]. High level of uric acid in the blood stimulates gout, a type of inflammatory arthritis caused by the deposition of monosodium urate crystal in synovial fluid and other tissues [23]. Xanthine oxidase (XO) is the key enzyme responsible for uric acid production by catalysis hypoxanthine into xanthine, and xanthine in turn into uric acid. It plays a vital role to cause hyperuricemia and gout [23,24]. To date, only allopurinol and febuxostat have been clinically approved as XO inhibitors to treat hyperuricemia and gout disease. However, due to negative effects of these XO inhibitors, such as hepatitis, nephropathy, and allergic reaction [25], new alternative natural products prepared in safer consumption, such as beverages with enhanced therapeutic properties and less side effects, are desired.

On the other hand, interests have also increased in finding naturally occurring antioxidants and antibacterial agents for use in food or medicinal materials from T. procumbens [27, 28]. Ethanol and methanol extracts of this plant noted to have antioxidant activity [13, 29]. Aqueous, ethanol, and methanol extracts of this weed possessed antibacterial activity against Escherecia coli [22,23], while its ethyl acetate extract showed antibacterial activity against Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, and Bacillus cereus [20].

However, bioactive constituents that responsible for biological activities in the plant have not been well examined. Thus, the present study was conducted to examine the xanthine inhibitory, antioxidant, and antibacterial activities of T. procumbens. The hot water extract of this plant relevant to these biological properties has also examined in order to evaluate potent utilization of this plant as a healthy source for the development of foods and beverages.

Medicinal properties of different extracts of Tridax procumbens recorded in various literatures [30]

Antimicrobial activity: The methanolic and ethyl acetate extracts of Tridax procumbens were tested against various bacterial species using Disc diffusion and Agar well diffusion methods. The ethyl acetate extracts were more effective than the methanolic extracts in both methods.

The ethyl acetate extract showed greater zone of inhibition against Staphylococcus aureus, Salmonella typhi and Bacillus cereus species, whereas, in the methanolic extract of Tridax procumbens, only Escherichia coli showed significant zone of inhibition, in disc diffusion method. In agar gel diffusion method,

Methanolic extract of Tridax procumbens showed antimicrobial activity for Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi and Escherichia coli and the ethyl acetate extract showed significant zone of inhibition against Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Escherichia coli and Bacillus cereus. Tridax procumbens leaf, extracted with ethyl alcohol is found to be most effective as an antimicrobial agent against Pseudomonas vulgaris. The ethanolic extract showed very good antibacterial activity against gram negative, non-fermenting multidrug resistant Pseudomonas isolated from nosocomial infections may be due to the presence of phyto constituents such as flavonoids and tannins have several mechanisms of action such as inhibition of DNA gyrase, inhibition of cytoplasmic membrane function, and inhibition of energy metabolism. The secondary metabolites have great therapeutic potential and also possess lesser side effects that are often associated with synthetic antimicrobial agents. The zone of inhibition tested for various gram negative organism, was well observed highest in Vibrio cholerae and lowest for Escherichia coli. The extract of Tridax procumbens L. was thus found effective against both Gram-positive and Gram-negative bacteria.

Anti- hepatotoxic or hepatoprotective activity: T. procumbens possibly activates muscarinic cholinergic receptors, which protects the liver via efferent vagus nerve. This plant is also used to prepare the drug "Bhringraj"; which is a reputed medicine in Ayurveda for liver disorders. The alcoholic extract of the plant is useful in liver regeneration; which has hepatoprotective action. The plant extract can improve the activity of liver antioxidant defense system, and it can repair the damage caused by free radicals. Rats pre-treated with chloroform insoluble fraction of ethanolic extract of Tridax procumbens has reversed the altered parameters like significant increase in the marker enzymes (aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase and gamma glutamyl transferase) and bilirubin towards normal due to the hepatitis induced by d- Galactosamine/Lipopolysaccharide (dGalN/LPS) . The ethanolic extract of T. procumbens has demonstrated its hepatoprotective action against CCl4 and the liver attained its normal appearance, similar to the liver cells of normal rats. The oral administration of varying doses of ethanolic extract of Tridax procumbens L.for the period of 7 days have also reversed the altered parameters to normal levels indicating the antioxidative and

hepatoprotective efficacy of Tridax. procumbens L. against paracetamol induced liver injury. Petroleum ether, methanol, and chloroform water extracts from flowers of Tridax procumbens showed protection against hepatotoxicity caused by D-galactosamine in male Wister albino rats, with the methanolic extract showing the best effect due to high phenolic contents. Aqueous extract of leaves have shown hepatoprotective activity in rats due to active free radical scavenging and antioxidant activity of the extract. [30]

Antifungal activity: The antifungal activity of T. procumbens may be due to the presence of many bioactive compounds such as, phenols, flavonoids, saponins, sterols and fatty acids.

The essential oils obtained from the flowers of Tridax procumbens L. were found to be active against the tested fungi . Methanol extract fractionated with dichloromethane have produced zones of inhibition ranging from 17 to 25 mm against various fungal strains including Microsporum fulvum (MTCC

8478), Microsporum gypseum (MTCC 8469), Trichophyton mentagrophytes (MTCC 8476), Trichophyton rubrum (MTCC 8477) and Candida albicans (MTCC 854). Among all other species C. albicans was highly susceptible. The antidermatophytic activity of the DCM fraction may be attributed to the presence of unsaturated fatty acids, 5-cholestane and different siloxanes Tridax procumbens also possesses antifungal property against three phytopathogenic fungi i.e. Helminthosporiumoryzae, Rhizoctonia solani and Pyricularia oryzae. The flowers also have excellent inhibitory potential against the tested plant pathogen, Fusarium oxysporum. Free flavonoids and sterols of T. procumbens (flower) have also completely inhibited the spore germination of the fungi.

Anti-cancerous activity: The in vitro anticancer activity of essential oil obtained from the leaves of T. procumbens was evaluated for MCF-7 cell lin by MTT assay, where the result revealed that the essential oil has significant anticancer activity which may be attributed to the presence of important terpenes like α -pinene and β -pinene. The flower crude aqueous and acetone extract of the plant Tridax procumbens was also tested on prostate epithelial cancerous cells PC3 by measuring cell viability by MTT assay. The assay was based on the capacity of mitochondrial enzymes of viable cells to reduce the yellow soluble salt MTT to purple blue insoluble formazan precipitate which was than quantified spectrophotometrically at 570 nm. The results of the analysis revealed anti-cancer activity of the crude flower extract.

Antidiabetic activity: Alpha amylase and alpha glucosidase are responsible for the hydrolysis of poly and oligosaccharides into monomers or cleavage of bonds between sugars and non- carbohydrate aglycone. These enzymes are involved in the digestion of carbohydrate into glucose or processing of the oligosaccharide moieties of glycoprotein. The methanolic extract of Tridax procumbens has the potential to reduce postprandial glucose levels via α -amylase inhibitory action. The retardation of membrane bound α -amylase inhibitory reaction or inhibition of passive glucose transports can flatten the postprandial blood glucose excursions or reduce hyperglycaemia. The alpha amylase activity of the methanolic extract of Tridax procumbens may be due to the presence of Quercetin. The administration of aqueous and alcoholic extracts from the leaves of Tridax procumbens (200 mg/kg) orally for 7 days produced a significant decrease in the blood glucose level in the alloxan-induced diabetic rat model. The ethanolic extract of the whole plant of T. procumbens also showed significant anti-diabetic and anti-hyperlipidemic activities against streptozotocin-induced diabetes in rats. Administration of ethanolic extract of the whole plant of T. procumbens to diabetic rats also resulted in an increase in their body weight. Flavonoids present in the plant regenerates the damaged beta cells of pancreases, and the polyphenolic compounds and saponin inhibits glucose transport by inhibiting sodium glucose co-transporter-1 (S-GLUT-1) in intestine .The methanolic extract of T. procumbens has shown better results than the standard drug Glibenclamide, against alloxan-induced diabetic male albino rats. The plant extracts were given to rats in 250 or 500 mg/kg doses, while the Glibenclamide was given at a 10 mg/kg dose. The results showed that both doses of the plant extract lowered the blood glucose levels in the rats better than the conventional drug after 6 hours of treatment. The plants extracts also improved the fasting blood glucose levels of the alloxan induced diabetic rats.

Repellency Activity: Essential oils extracted from leaves of Tridax procumbens Linn. by steam distillation after examination for its topical repellency effects against malarial parasite Anopheles stephensi in mosquito cages at three different concentrations (2%, 4% and 6%), exhibited relatively high repellency effect (>300 minutes at 6% concentration). The water and ethanol decoctions also reported to have anti-plasmodial properties against chloroquine- resistant Plasmodium falciparum

Anti-urolithiatic activity: Renal calculi formation is one of the common urological disorders. Hyperoxaluria and hypercalciuria are the major risk factors for renal stone formation. Ethanolic extract of T. procumbens was evaluated against 0.75% v/v ethylene glycol and 2% w/v ammonium chloride induced calcium oxalate urolithiasis and hyperoxaluria induced oxidative stress in male albino rats. Treatment with the extract reduced calculogenesis and renal deposition of calcium and oxalate and resultant lipid peroxidation, indicating its antiurolithiatic and antioxidant effects. Thus T.procumbens has proven its efficacy to be useful in the treatment of renal stone disease.

Wound healing activity: The whole Plant Extract (WPE) of Tridax procumbens demonstrated greatest pro-healing activity as evidenced by the increase in tensile strength and lysyl oxidase activity after being studied on a dead space wound in albino rat. Granuloma tissue harvested from10 day old wounds was used for estimation of lysyl oxidase activity, tensile strength and other biochemical parameters. The aqueous extract was also seen to be effective in increasing lysyl oxidase activity but to a lesser extent than WPE. The pro healing action of the plant may be attributed to the presence of fumaric acid. The aqueous and ethanolic extracts of the whole plant of Tridax procumbens Linn. were also evaluated for the wound

healing activity. The ethanolic extract was quite more effective in increasing wound contraction compared to the aqueous extract. The topical application of ethanolic extract of the plant showed significantly higher tensile strength than the aqueous extracts, and the standard drug, cipladine control groups also showed much lesser tensile strength than the extracts treated groups. Both the extracts of the plant have not only increased granulation and hexosamine formation but also, showed significant increase in hydroxyproline content of the granulation tissue which indicated rapid collagen formation. Tridax procumbens Linn. may become a useful component for healing the wounds.

Anti-hypertensive activity: Vasodilatation can be facilitated by inhibition of vasoconstriction and secretion of relaxant factors from vascular endothelium. T. procumbens leaves have been reported to contain several active compounds such as alkaloids, flavonoids, quercetin, arachidic, and linoleic acid. Quercetin has been known to decrease Blood Pressure (BP) and/or reduced the severity of hypertension in spontaneously hypertensive rats. The flavonoid luteolin has also induced NO production and arterial relaxation. The aqueous extract of the leaves of T. procumbens was evaluated for assessing their relaxation effect in the aortic artery that was pre- contracted with Phenylephrine (PE) and KCl by the mechanistic interactions with Nitric Oxide (NO) synthase, cyclic Guanosine Monophosphate (cGMP), and cyclic Adenosine Monophosphate (cAMP). The results showed that the TPE significantly reduced the contraction induced by PE in a concentration-dependent manner. Apart of the relaxing effect of Tridax is mediated directly by blocking or modulating cGMP and Camp The effect of Tridax procumbens Aqueous Leaf Extract (TPALE) was also investigated on reproductive function in N nitro-L-arginine-methyl ester (L-NAME) induced hypertensive rats. The results proved that TPALE decreased systolic, diastolic and mean arterial blood pressure in L-NAME+TPALE treated groups compared to only L-NAME treated group

Anti-inflammatory activity: The most active fraction of T. procumbens responsible for anti- inflammatory activity is Ethyl Acetate (ETA) fraction as it was found to contain moderate polar natural products like alkaloids and flavonoids. The alkaloids and flavonoids can counteract Reactive Oxidative Species (ROS) involved in the pathogenesis of inflammation and related ailments in biological systems. Tridax procumbens leaves were tested for their contractile activity in response to the potent gastrointestinal constrictors .Oral exposure of Aqueous Tridax procumbens Leaf Extract

(ATPLE) to the adult male wistar rats potentiated the contraction in duodenal and jejunal small intestinal smooth muscle. Contraction in response to M3 receptor subtype activator (acetylcholine) which couples to Gq and PKC, H1 receptor subtype activation (histamine) and high conductance Ca2+ activated K+ channel activator (KCl) was significantly enhanced in ATPLE treated group as compared to control group. Thus, the enhancement in the contraction of ATPLE treated rats may be predicated on Tridax procumbens ability to offer protection against inflammation and tissue damage to gastrointestinal smooth muscle.

Hemostatic activity: Various extracts like ethanolic extract, fresh leaf and petroleum extract of the leaves of Tridax procumbens were screened for hemostatic activity by studying the clotting time of 10 human volunteers using Lee White's method performed in vitro. Among them the ethanolic extract had positive activity by reducing the clotting time uniformly in the blood samples of all the subjects. Aqueous leaf extract have also shown enhanced blood clotting activity, thus it may be used as a potent haemostatic agent.

Anti-diarrheal activity: The aqueous and ethanolic leave extract of Tridax procumbens was also evaluated for their antidiarrheal activity on gastrointestinal motility with barium sulphate milk model and the castor oil induced diarrheal model. Both the aqueous and ethanol leave extracts of Tridax procumbens showed significant antidiarrheal activity on gastro intestinal motility with barium sulfate milk model but, the aqueous extract showed no significant reduction in the number of wet faeces for almost 2 hours compared with the standard lomotil drug on the castor oil induced diarrheal model.

Leishmanicidal activity: In vitro activity of methanolic extract of T. procumbens inhibited promastigotes growth of Leishmania mexicana which is a causative agent of cutaneous leishmaniasis disease with a 50% Inhibitory Concentration at 3 μ g/ml, showing its anti- leishmanial activity.

MATERIALS AND METHODS

Plant Material

The plant was collected from nearby region. After authentication, fresh stems material was collected in bulk, washed, shade dried and pulverized in mechanical grinder to obtain coarse powder.

Extraction Methodology

The root coarse powdered material (each 100 gm) was soaked in ethanol, ethyl acetate, chloroform, water (i.e. Maceration technique) for continuous 72 hours and then filtered off. Following extraction, the liquids were concentrated to remove traces of solvents and the process was repeated for two times. The solvent from total extract was distilled off and concentrate was evaporated on water bath to syrupy consistency and then evaporated to dryness. So our specimen was prepared.

Worms Collection

Indian earthworm Pheretima posthuma (Annelida) [31] were used to study anthelmintic activity. The earthworms were collected from the moist soil from the near region of Jamner, Jalgaon, and washed with water. The earthworms in 3-5 cm

in length and 0.1-0.1-2 cm in width were used for all experimental protocol. The earthworm resembles both anatomically and physiologically to the intestinal roundworms parasites of human beings, hence can be used to study anthelmintic activity.

RESULTS AND DISCUSSION

The preliminary phytochemical investigation of all the extracts of Tridax Procumbens shows presence of steroids, tannins, alkaloids, flavonoids, carbohydrates, alkaloids, glycosides. Some of these phyto- constituents may be responsible to show a potent anthelmintic activity. The perusal of the data reveals that as the concentration increases paralysis and death time decreases. In this study the paralysis time and death time of the earthworms in different doses of extract (20mg/ml, 40mg/ml and 60mg/ml) were determined. Albendazole drug at concentration (20mg/ml) were used as standard/reference drug to compare anthelmintic activity of the ethanolic extract of Tridax Procumbens Stem against Indian earthworm.

Sr.no	Concentration (mg/ml)	Time For Paralysis	Time For Death
		(min)	(min)
1	Control(normal aline)		
2	Albendazole (20mg/ml)	17	24
3	Ethanolic extract 20mg/ml	33	49
4	Ethanolic extract 40mg/ml	25	40
5	Ethanolic extract 60 mg/ml	18	28



TABLE: Anthelmintic Activity of ethanolic extract of Tridax Procumbens.

The above graph represent the Anthelmintic activity of Albendazole (std. Dose) and ethanolic extract of Tridax Procumbens stem against Indian earthworm. In this study the paralysis time and death time of the earthworms in different doses of the extracts (20mg/ml, 40mg/ml, and 60 mg/ml) were determined. Albendazole drug at concentration (20mg/ml) were used as standard/reference drug to compare anthelmintic activity of the ethanolic extract Tridax Procumbens stem against Indian earthworm. The paralysis time and death time of the worms in different doses of the extract were noted shown in the table no.1.The control there is no death and paralysis of Indian earthworm.



The standard dose of Albendazole drug at concentration 20 mg/ml show paralysis time and death time at 17 and 24 min. respectively were used as reference. The ethanolic extract of Tridax Procumbens (stem) concentrations at 60 mg/ml show paralysis time and death time at 18 and 28 min. From the result it concluded that, ethanolic extract of Tridax Procumbens (stem) were show concentration dependent anthelmintic activity.

CONCLUSION

The Anthelmintic activity of Tridax Procumbens stem (extract) shows better activity on helminth causing worms with increasing concentration of the extract. From this research work, it found that ethanolic extract of Tridax Procumbens stem were show concentration dependent anthelmintic activity. Further studies need to isolate and reveal the active compound in the crude extracts of Tridax Procumbens stem and establish the mechanism of action are required.

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