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A Review of Anti microbial activity and phytochemical screening of leaf extracts of Tridax procumbens Linn

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ABSTRACT: Tridax procumbens Linn belongs to the family Compositae. The extracts of Tridax procumbens have been reported to have various pharmacological effects like antibacterial, mosquito repellent activity, leishmanicidal, hepatoprotective effect on liver antioxidant system, immunomodulatory effect, wound healing activity and antiprotozoal effects.It is commonly called as Coat button in English or Ghamra in Hindi.Phytochemical screening of Tridax leaf extracts reveals that it contains steroid,tannin,saponin, alkaloids, protein, amino acids,diterpenes,phytosterol,phenol, flavonoids and cardial glycosides .Leaf extract are prepared by using different solvents.Antimicrobial activity is found by using different screening methods.

KEYWORDS: Tridax procumbens Linn, soxhlet apparatus, phytochemical screening,zone of inhibition.

I. INTRODUCTION

Traditional medicine has expanded worldwide and is popular. Plants have been used for thousands of years in India and other countries. Herbal medicines serve about 80% of the world's population health needs for millions of people. Amongst these, the plants with antimicrobial potential has become the need of today's research. The side effects and toxicities of synthetic antibiotics justify the need to search for new antimicrobial agents from plant source .Tridax procumbens (family-Asteraceae) is a perennial plant. They are available in all seasons. It has been known by several names like coat buttons in English, ghamra in Hindi, Jayanti veda in Sanskrit, herbe caille in French, vettukaaya poondu in Tamil. It is a weak straggling herb about 12-24cm long with few leaves 6-8cm long and grows on roadsides, hedges and in wastes globally. The leaves of this plant including other aerial parts except flowering tops have been claimed to be useful in the treatment of inflammatory conditions and have tendency to heal wounds, anti-diabetic activity, anti-arthritic activity, prevent hair loss, diarrhoea and serve as insect repellent[1].

One of the leading causes of death throughout the world is infectious diseases and the resistance has grown within the infectious organisms against antibiotics. In addition, antibiotics used sometimes show adverse effects including hypersensitivity reactions, immune suppression and allergic reactions. To combat this problem, there is a continuous search for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases . The natural compounds obtained from plants can provide potential means for the development of new drugs .

T. procumbens (L) is a common medicinal herb belonging to family Asteraceae and is used by ethno-medical practitioners as antifungal, antimicrobial agents.The tribal peoples in some Indian states uses leaf juice to cure fresh wounds, as a hair tonic and to stop bleeding[2**].**

DRUG PROFILE

T. procumbens (fig.1)is found in tropical and subtropical areas of the world growing with annual crops, along roadsides, pastures, fallow land, and waste areas . The species has a diploid number of 36 . It has herbaceous, semi-prostrate habit, and can with grow anywhere from 15-40 cm in height. The leaves are elongated, opposite, ovate serrated margins, hirsute on the abaxial and adaxial side . The inflorescence is a capitulum with three-toothed white ligulate ray florets female and disc inner flowers yellow, tubular, bisexual, with corolla 6 mm long. The inflorescence results in abundant production of pappus achenes ,2 mm long, obovoid, setaceous, covered with stiff hairs, that

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can be carried by the wind for long distances, making this species a potential invasive species if not controlled [3**].**

Botanical classification of Tridax[4]

Kingdom : Plantae
Sub-kingdom : Tracheobionta
Division : Magnoliophyta
Class : Magnoliopsida
Sub class : Asteridae
Order : Asterales
Family : Asteraceae
Genus : Tridax L
Species : Tridax procumbens (L)

Figure.1 Tridax procumbens Linn

CHEMICAL COMPOSITION:

Flavonoid (procumbenetin) isolated from the aerial parts of Tridax procumbens has been characterized as 3, 6 –dimethoxy-5, 7, 2', 3', 4'-pentahydroxy flavone 7-0-β-D-glucopyranoside1 on the basis of spectroscopic techniques & by chemical means.Isolation of methyl 14 oxobutanoate,methyl 14- oxononanoate, 3-methyl-non decylbenzene, heptacosanol cyclohexane carboxylate, 1-(2,2, dimethyl-3-hydroxy propyl)-2-isobutyl phthalate,12-hydroxytetracosa-15-one,32-methyl-30-ozotetraatriacont-31-en-1-ol along with β amyrin, β amyrone, fucosterol & sitosterol, arachidic, behenic, lauric, linoleic, linolenic, myristic, palmitic & stearic acids have been isolated.It is also a potential source of the protein supplements & pro vitamin A (carotenoid) **[4].**

T. procumbens contains flavone glycosides, chromone glycosides, sterols and polysaccharides with a Beta-1,6-D-galactan main chain. Unsaponifiable fraction of petroleum ether fraction revealed the presence of campesterol, stigmasterol and beta- sitosterol by GC-MS .The ethyl acetate soluble part of hexane extract yielded a new bithiophene named tri-bis bithiophene along with four terpenoids: taraxasterol acetate, beta-amyranone, lupeol and oleanolic acid [5].

PHARMACOLOGICAL PROPERTIES:[4]

1. Hepatoprotective activity:

Its hepatoprotective action was seen in d-Galactosamine/Lipopolysaccharide(d-GalN/LPS) induced rats. d-GalN/LPS are hepatotoxic by its action of destroying liver cells. It selectively blocks the transcription & indirectly hepatic protein synthesis causing endotoxin toxicity & leading to fulminant hepatitis within 8 hrs of administration.The results revealed that T. procumbens could afford a significant protection in the alleviation of d-GalN/LPS-induced hepatocellular injury.

2. Immunomodulatory activity

Albino rats dosed with Pseudomonas aeruginosa when administered with ethanolic extract of leaves of Tridax showed stimulation of humoral immune response along with elevation in hemagglutination antibody titer. It also inhibited proliferation of P. aeruginosa along with significant increase in phagocytic index, leukocyte count & splenic antibody secreting cells.

3. Wound healing activity

Tridax opposed anti epithelization & tensile strength depressing effect of dexamethasone (a well known healing suppressant agent) without affecting anti contraction & anti granulation action of dexamethasone. Aqueous extract was also effective in increasing lysyl oxidase, but to a lesser degree than whole plant extract. Further, it has been shown that extract of leaves of T. procumbens promotes wound healing in both normal & immunocompromised (steroid treated) rats in the dead space wound healing model. but also The plant increases not only lysyl oxidase, protein & nucleic acid content in the granulation tissue, probably as a result of increase in glycosaminoglycan content.

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4. Antidiabetic activity

Aqueous & alcoholic extract of leaves of Tridax Showed a significant decrease in the blood glucose level in the model of alloxan induced diabetes in rats.

5. Anti microbial activity

Whole plant of Tridax has been reported for its anti microbial activity on various species of bacteria. Fresh plant juice when applied twice a day for 3-4 days cures cuts & wounds. Whole plant extract when used against 4 strains of bacteria – two gram positive-Bacillus subtilis, Staphylococcus aureus & two Gram negative Escherichia coli & P. aeruginosa showed anti bacterial activity only against P. aeruginosa.Tridax procumbens also possess antifungal property against three phytopathogenic fungi i.e.Helminthosporium oryzae, rhizoctonia solani & pyricularia oryzae.Methanolic extract of leaves of T.procumbens were found to be active against two tested fungi (A. niger and A. ochraceous). The fungal strain of A. niger and A. ochraceous shows zone of inhibition 13mm and 12mm respectively where positive control (ciprofloxacin) produced zone of inhibition 11mm and 10mm respectively.The n-hexane extract of the flower showed activity against E. coli. The same extracts of the whole aerial part were active against Mycobacterium smegmatis, E-coli, Salmonella paratyphi & Staphylococcus aureus while aqueous extract showed no antimicrobial activity. Among the various karmas defined of Tridax procumbens, it's antimicrobial action, in present era when man is surrounded by countless microorganism & human body has become resistant to many of the strains of bacteria & fungi, has emerged as a new ray of hope.

6. Defluoridation action

Fluoride though acts as a protective agent for teeth but when in excess it is harmful to health. Recently, researchers in India have developed a filter system based on medicinal herbs, which can quickly & easily remove fluoride from drinking water. T. procumbens, a medicinal herb in India, previously was tested for extraction of toxic heavy metals from water. Singanan has suggested that this medicinal herb can be used as a biocarbon absorbent for fluoride. He explained that by loading up plant tissue with aluminium ions, it is possible to create a safe bio carbon filter that will readily absorb fluoride ions from water warmed to around 270 C passing through filters. His trial also showed that it takes just 3 hrs to remove 38% of fluoride with just 2g of the bio carbon filter. So this bio carbon filter might provide an inexpensive way to defluoridate water in regions where the natural level of this mineral is high in groundwater including China, India, Sri Lanka, Italy, Spain, Mexico, Holland, West Indies, North & South America.

7. Antiviral activity

The therapeutic potential of T. procumbens L. extracts were screened for antitrypanosomal properties in mice infected with Trypanosoma brucei by Abubakar et al & found insufficient anti trypanosomal activity, though stated that the modification of the detected phenolic compound may generate effective antitrypanosomal drug.

8. Anti inflammatory activity

The anti-inflammatory action of leaf extract of Tridax was assessed on carrageenan induced paw edema along with standard drug, Ibuprofen. The extract increased the inhibition of oedema if treated with standard drug Ibuprofen. Water soluble powder of leaf extract was administered orally at different doses to rats. The result demonstrated that the extract possess analgesic activity. T.procumbens dose reduced the abdominal writhing.Meshram & Patel investigated that alcoholic & hydroalcoholic extracts have anti inflammatory activity using the rat paw oedema assay & showed oedema inhibition 0.82%, 16.80%, 11.39%.

9. Antiurolithiatic activity

Ethanolic extract of Tridax procumbens L. was used for treating kidney stone disorders. It was evaluated against 0.75% v/v ethylene glycol & 2% v/v ammonium chloride induced calcium oxalate urolithiasis & hyperoxaluria induced oxidative stress in male albino rats. Treatment with the extract was able to reduce calculogenesis induced urinary excretion & renal deposition of calcium oxalate & resultant lipid peroxidation including its antiurolithiatic & antioxidant effect.

10. Hypotensive

Cardiovascular effect of aqueous extract of T.procumbens leaf was tested on anaesthetized Sprague Dawley rat. The aqueous extract caused a significant dose dependent decrease in mean arterial blood pressure. The higher dose leads to significant reduction in heart rate whereas lower dose did not cause any change in the same. Thus leaves of T. procumbens showed hypotensive effects.

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11. Repellent activity

In a study, essential oils were extracted by steam distillation from leaves of T. procumbens L. & were examined for its topical repellency effects against malaria parasite Anopheles stephensi in mosquito cages.All essential oils were tested at three diff. concentration (2, 4 & 6 %) of these, the essential oils of Tridax exhibited relatively high repellency effect (>300 minutes at 6% conc. & calculated that Tridax are promising as repellant at 6 % conc. against A stephensi.

12. Anticancer activity

The activity of T. procumbens flower crude aqueous & acetone extract was tested on prostate epithelial cancerous cells. PC3 was determined by measuring cell viability by MTT assay .Experiment consists of cleavage of the soluble yellow coloured tetrazolium salt MTT [3-4, 5- dimethyl-thiazole-2-yl)-2,5diphenyl-tetrazolium bromide] to a blue coloured formazan by the mitochondrial succinate dehydrogenase. 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 is the quantified spectro photometrically at 570 nm.

13. Antioxidant property

Antioxidants prevent the damage done to cells due to free radical molecules released during normal metabolic processes. The results of DPPH radical scavenging activity of Tridax against test sample & standard (gallic & ascorbic acids (Fluka) shows that

Tridax possesses a very high percentage of antioxidant activity, 96.70% at a concentrator of 250 µg/ml. It shows a reductive potential of 0.89 mm. Tridax extracts may have hydrogen donors thus scavenging the free radical DPPH with High AA% of 96.70% at 250 µg/ml which was observed to be higher than those of standards (ascorbic & gallic acids) at a conc. of 250 µg/ml used. Thus Tridax plants are a rich source of natural antioxidants.[4]

EXTRACTION:

Flowers of Tridax procumbens plant were collected and kept for shed drying for optimal period. After complete drying they were subjected to grinding into coarse powder by mechanical means. The powdered flowers were kept for maceration in methanolic medium for 96 Hrs at room temperature and filtered **[**6**].**

Plant material was washed thoroughly with tap water and shade dried at room temperature. The leaves were powered in an electronic blender to obtain coarse powder of leaves. It was passed through sieve no. 40 and then stored in a closed container at room temperature for further use. The extraction procedure was carried out in soxhlet apparatus (fig.2) with 100 grams of coarse powder of the leaves of Tridax procumbens using hydro-alcohol (500ml), pet-ether (500 ml) and distilled water (500ml) separately for 24 hrs and filter. The concentrated extract was then evaporated to dryness in a vacuum oven at temperature 40°c. The dried extract was stored at 4°c in an air free sterile container in the refrigerator for preliminary phytochemical analysis[7**].**

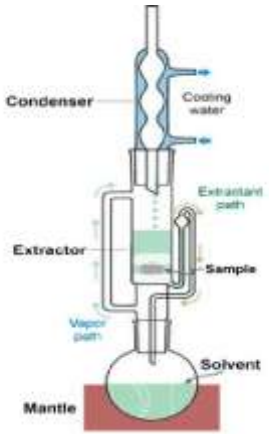

Figure.2.soxhlet apparatus

PRELIMINARY PHYTOCHEMICAL SCREENING:

Phytochemical analysis can be carried out in petroleum ether,benzene, chloroform, alcohol and

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aqueous extract of leaves, stem and callus of Tridax procumbens using standard procedures.

Proteins:

Xanthoproteic test: Extract was treated with few drops of concentrated HNO3 formation of yellow indicates the presence of proteins.

Biuret test: To 3 ml of the extract few drops of 10% NaCl and 1% copper sulphate was added for the formation of violet purple colour.

Millon's test: To 3ml of the extract few drops of Millon's reagent was added for the formation of red colour.

Amino acids :

Ninhydrin test: To the 2 ml extract 2 ml on ninhydrin reagent was added & boiled for few minutes, formation of blue colour indicates the presence of amino acid.

Carbohydrates:

Molisch test: To a small amount of the extract few drops of Molisch reagent was added followed by the addition of conc. Sulphuric acid. The mixture was then allowed to stand for 2 min and then diluted with 5ml of distilled water. Formation of red or violet colour at the inter phase of two layers indicates the presence of carbohydrates.

Fehling's test: The extract was treated with 5ml of Fehling's solution (A and B) and kept in a boiling water bath. The formation of yellow or red colour indicates the presence of reducing sugar.

Flavonoids :

Shinoda' S Test- To 2 ml of the test solution a piece of Mg++ ribbon and concentrated HCl was added drop by drop. The resulting pink/ scarlet crimson or occasionally green/blue colour indicated the presence of flavonoids.

Alkaloids :

Each of the test samples was acidified with 2% HCl heated (600) for 2 h which was later, cooled and filtered. A formation of white precipitate in addition of 2-3 drops of following reagents to 2 mL of the above solution indicated the presence of alkaloid.

1. Modi Mayer's reagent- Prepared by mixing 1.35g HgCl2 and 3.95 g KI in 100ml of distilled water.

2. Wagner's reagent- Prepare by mixing 1.27g I2 and 2.00 g KI in 100 ml distilled water.

3. Bourchardt's reagent- Prepare by mixing 2.0g I2 and 4g KI in 100 ml distilled water**[8].**

saponin:

Two grams of crude ethanol extract of Tridax procumbens leaf was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of filtrate of ethanol extract of Tridax procumbens leaf was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing solution was mixed with 3 drops of olive oil and shaken vigorously. The solution then formed an emulsion indicating the presence of saponin.

steroids: Two milliliters of acetic anhydride was added to 0.5 g of crude ethanol extract of Tridax procumbens leaf with 2 ml of concentrated H2S04. The solution turned light green indicating the presence of steroids.

cardiac glycosides (keller-killani test): One gram of crude ethanol extract of Tridax procumbensleaf was dissolved in 5ml distilled water, 5ml of the dissolved crude ethanol extract of Tridax procumbens leaf was treated with 1ml of glacial acetic acid with one drop of ferric chloride solution. 1ml of concentrated H2S04 was later added. A brown ring of the interface was observed indicating the presence of a deoxy-sugar. A violet ring appeared below the brown ring on the acetic acid layer, the absence of greenish blue solution indicates the absence of cardiac glycosides

Tannin: One gram of crude ethanol extract of Tridax procumbens leaf was dissolved in 5ml distilled water. 5ml of the dissolved crude ethanol extract of Tridax procumbens leaf was dissolved in 1ml of 5% ferric chloride the solution formed greenish-black precipitate indicating the presence of tannin**[**9**].**

Test	Ethanol	Chloroform	Aqueous
Glycosides	+	-	+
Flavonoids	+	+	+
Saponins	-	-	+

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Steroids	-	-	-
Alkaloid	-	+	-
Carbohydrates	+	-	-
Polyphenol	+	-	+
Tannins	+	+	-

Table.1 physicochemical analysis of leaf extract of Tridax procumbens using different solvents [10].

SCREENING OF ANTIMICROBIAL ACTIVITY:

Antimicrobial susceptibility test (AST) is used to determine the efficacy of potential antimicrobials from natural products against a number of microorganisms. In clinical research, in vitro susceptibility tests are important if an organism is suspected toward resistance to frequently used antimicrobial agents.Evaluation of the performance of a susceptibility test should have ease of use, reproducibility, test sensitivity and specificity. AST standard tests can be conveniently divided into diffusion and dilution methods. Common diffusion tests include agar well diffusion,agar disk diffusion and bioautography, while dilution methods include agar dilution and broth micro or macrodilution.

Antibacterial assays Methods which are used to evaluate the activity of plant antimicrobials are divided into in vitro and in vivo (application test). The former may be termed "screening methods" and might include any test in which the compound is not applied directly to the product under use conditions. Generally, these tests provide preliminary information to determine potential usefulness of the test compound. The second type includes those tests in which an antimicrobial is applied directly to a product **[**11**].**

Agar disc-diffusion method:

Agar disc-diffusion testing developed in 1940 , is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts testing. Although not all fastidious bacteria can be tested accurately

by this method, the standardization has been made to test certain fastidious bacterial pathogens like streptococci, Haemophilus in-fluenzae, Haemophilus parainfluenzae, Neisseria gonorrhoeae and Neisseria meningitidis, using specific culture media, various incubation conditions and interpretive criteria for inhibition zones.In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface.The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured Nevertheless, disk-diffusion assay offers many advantages over other methods: simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease to interpret results provided **[**12].

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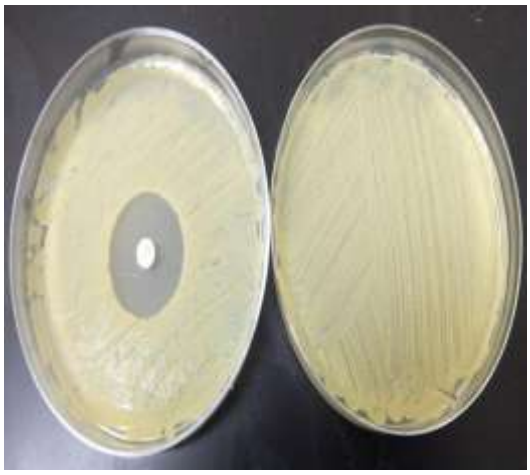

Figure.3 zone of inhibition using Agar disc diffusion method.

Agar well diffusion method:

Agar well diffusion method is widely used to evaluate the an-timicrobial activity of plants or microbial extracts. Similarly to the procedure used in disk-diffusion method, the agar platesurface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface.Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 mL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then,agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested [13**].**

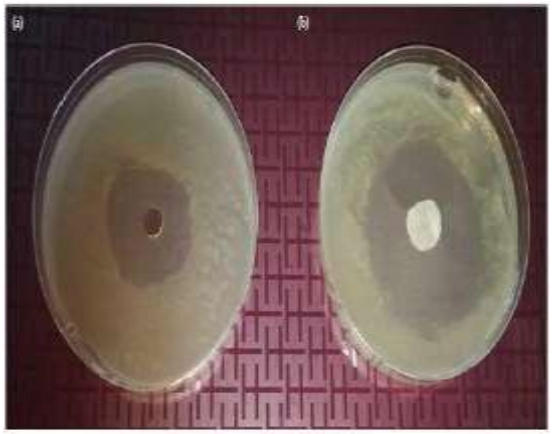

Figure.4 zone of inhibition using Agar well diffusion method.

CONCLUSION:

Tridax procumbens is the plant which is easily available in rural areas.They are traditionally used as folk medicine.In present, microbes develop resistance against antibiotics and there is a need to develop new antibiotics. By further investigating these plants ,it may lead to identification of new constituent which can be used in drug development for human use. Tridax can be combined with other naturally occurring plant drug for synergistic effect. In future, a lot of researches should be conducted on various pharmacological properties of Tridax procumbens Linn and it should be useful for preparation of new drugs and for preventive medicine.

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