The ethanolic extract of Tridax procumbens, linn (Asteraceae) (commonly known as Coat Buttons) were evaluated for antimicrobial activity and antifungal activity. The dried powder of Tridax procumbens (leaves, stems, roots and flowers) containing chemical constituent procumbent was extracted and the activity was studied. Various concentration (5 mg/ml, 10 mg/ml and 15 mg/ml) of ethanol were evaluated to study the activity against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Aspergillus flavus, Candida albicans. The antibacterial activity was measured by agar well diffusion method and antifungal activity by disc diffusion method. Tridax procumbens (15 mg/ml) showed maximum zone of inhibition was against Gram positive bacteria Staphylococcus aureus (18mm) and minimum against Gram negative bacteria Escherichia coli (8mm). Tridax procumbens (15mg/ml) showed maximum antifungal activity towards Aspergillus flavus (12mm) and Candida albicans (4mm). The results showed significant activity of Tridax procumbens and suggesting its use as natural antimicrobial agent.

FOR AUTHOR USE ONLY

Mrs. Vishin Ashish Patil, M. Pharm (Pharmacognosy) is presently working as Lecturer at Dr. J. J. Magdum Trust's, Anil Alias Pintu Magdum Memorial Pharmacy College, Dharangutti, Tal- Shirol, Dist- Kolhapur, Maharashtra, India. She has 14 years of academic experience. She has published 14 papers in international journals.

9 786205 487549

Vishin Patil
Sachin Nitave

ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF TRIDAX PROCUMBENS LINN

ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF TRIDAX PROCUMBENS LINN ON DIFFERENT PATHOGENS ITS PHYTOCHEMICAL SCREENING

LAP LAMBERT Academic Publishing

Vishin Patil
Sachin Nitave

ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF TRIDAX PROCUMBENS LINN

FOR AUTHOR USE ONLY

FOR AUTHOR USE ONLY

Vishin Patil
Sachin Nitave

ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF TRIDAX PROCUMBENS LINN

ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF TRIDAX PROCUMBENS LINN ON DIFFERENT PATHOGENS ITS PHYTOCHEMICAL SCREENING

FOR AUTHOR USE ONLY

LAP LAMBERT Academic Publishing

Imprint
Any brand names and product names mentioned in this book are subject to trademark, brand or patent protection and are trademarks or registered trademarks of their respective holders. The use of brand names, product names, common names, trade names, product descriptions etc. even without a particular marking in this work is in no way to be construed to mean that such names may be regarded as unrestricted in respect of trademark and brand protection legislation and could thus be used by anyone.

Cover image: www.ingimage.com

Publisher:
LAP LAMBERT Academic Publishing
is a trademark of
Dodo Books Indian Ocean Ltd., member of the OmniScriptum S.R.L Publishing group
str. A.Russo 15, of. 61, Chisinau-2068, Republic of Moldova Europe
Printed at: see last page
ISBN: 978-620-5-48754-9

Copyright © Vishin Patil, Sachin Nitave
Copyright © 2022 Dodo Books Indian Ocean Ltd., member of the OmniScriptum S.R.L Publishing group

FOR AUTHOR USE ONLY

ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF TRIDAX PROCUMBENS LINN WHOLE PLANT ETHANOLIC EXTRACT ON DIFFERENT PATHOGENS AND ITS PHYTOCHEMICAL SCREENING

ACKNOWLEDGEMENT

Research is a never ending process involving a team of persons striving to attain newer horizons in the field of sciences. This study would not have been completed without the encouragement and co- operation of my teachers, parents, friends, well-wishers and relatives. I take this opportunity to express my deep gratitude to one and all.

My heartful gratitude to Hon. Dr.Veejhay Magdum, Chairman, Dr. J. J. Magdum Trust, Jaysingpur and Hon. Adv. Dr. Sonale Veejhay Magdum for their support and encouragement.

I am very much thankful to Dr Sachin Annasaheb Nitave Principal Dr. J. J. Magdum Trust's Anil Alias Pintu Magdum Memorial Pharmacy college, Dharagutti, Maharastra for his guidance and valuable support.

"A parent's love is whole no matter how many times divided. I am very much thankful to my dear parents, my mother Smt. Sharda Madhukar Eknath for their affectionate support for my research work. I extend my heartfelt gratitude to my in laws specially my mother in law Smt. Swati Tatyasaheb Patil for their support throughout the entire work. I have no words to express my gratitude to my beloved Husband Mr. Ashish Tatyasaheb Patil for his inspiration throughout my studies and unconditional support. I thank to dear Son Master Udayan Ashish Patil for this affection and support.

FOR AUTHOR USE ONLY

TABLE CONTENTS

FOR AUTHOR USE ONLY

Abstract

The ethanolic extract of Tridax procumbens, linn (Asteraceae) (commonly known as Coat Buttons) were evaluated for antimicrobial activity and antifungal activity. The dried powder of Tridax procumbens (leaves, stems, roots and flowers) containing chemical constituent procumbent was extracted and the activity was studied. Various concentration (5 mg/ml, 10 mg/ml and 15 mg/ml) of ethanol were evaluated to study the activity against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Aspergillus flavus, Candida albicans. The antibacterial activity was measured by agar well diffusion method and antifungal activity by disc diffusion method. Tridax procumbens (15 mg/ml) showed maximum zone of inhibition was against Gram positive bacteria Staphylococcus aureus (18mm) and minimum against Gram negative bacteria Escherichia coli (8mm). Tridax procumbens (15mg/ml) showed maximum antifungal activity towards Aspergillus flavus (12mm) and Candida albicans (4mm). The results showed significant activity of Tridax procumbens and suggesting its use as natural antimicrobial agent. Ampicillin was used as standard antibacterial drug Normal saline solution and alcohol was used as control to study antimicrobial activity and Amphotericin B was used as antifungal drug to study antifungal activity. The results of present study indicated that ethanolic extract of Tridax procumbens linn shows has potent antimicrobial and antifungal activity.

FOR AUTHOR USE ONLY

Keywords: Tridax procumbens, Antibacterial, Antifungal activity, S.aureus, E.coli, P.aeruginosa.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources. Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents. Emergence of pathogenic microorganisms that are resistant/ multi resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs. In addition, high cost and adverse side effects are commonly associated with Popular synthetic antibiotics (such as hypersensitivity, allergic reactions, immjuno suppression etc.) and are major burning global issues in treating infectious diseases. Although pharmacological industries had produced considerable number of commercial antibiotics time to time but resistance in pathogens towards these drugs too has increased at high rate and multi drug resistant microorganisms have exacerbated the situation. In the present scenario, there is an urgent and continuous need of exploration and development of cheaper, effective new plant based drugs with better bioactive potential and least side effects. Hence, recent attention has been paid to biologically active extracts and compounds from plant species used in herbal medicines. Antimicrobials of plant origin have enormous therapeutic potential and have been used since time immemorial. They have been proved effective in the treatment of infectious diseases simultaneously mitigating many of the side effects which are often associated with synthetic antibiotics. Positive response of plant based drugs (less/ no side effects) might lies in the structure of the natural products which reacts with toxins and/or pathogens in such a way that less harm is done to other important molecules or physiology of host. It is because of this reason that drug designing studies nowadays have come up as new field of research. Man always been surrounded by countless microorganisms. The disease producing microbes are playing a very important role in human life. Pathogenic

FOR AUTHOR USE ONLY

microorganisms are always trying to develop resistance to the various antimicrobial agents used for their control. Therefore, the chemotherapy of communicable diseases has proved to be a continuous great effort. Scientists are forever in exploring of new antimicrobial agents to run the ever increasing menace of the microbes. Thus it is of overriding importance for the microbiologists to develop new resistant strains. Therefore, medicinal plants are gifts of nature to cure limitless number of diseases among human beings. Tridax procumbens linn is commonly known as 'Ghamra' in Hindi and 'Dagadi Pala'in Marathi. It is a weed found throughout India. A hispid, procumbent herb with woody base sometime rooting at the node, up to 60 cm high. Leaves are ovate-lanceolate 2 to7 cm and lamina pinnatisect, sometimes three lobed, flowers in small, long peduncled heads. It is commonly used in Indian traditional medicine as anticoagulant, antifungal and insect repellant, in bronchial catarrh, diarrhea and dysentery. Moreover it possesses wound healing activity and promotes hair growth. The leaf gel possesses antiseptic, insecticidal and parasiticidal properties.The plant also shows various pharmacological activities like Immunomodulatory, Antidiabetic, Anti hepatotoxic & Anti-oxidant, Anti-inflammatory, Analgesic, and marked depressant action on respiration. Aqueous leaves extract possess cardiovascular effect and significantly reduces heart rate and blood pressure. Lyophilized aqueous leaf extract showed anti-inflammatory action comparable to ibuprofen and aspirin. Whole arial parts have hepatoprotective, antisecretory (antidiarrhoeal) activity. It is active against bacteria, protozoa and fungi. Leaf juice is useful in dead space wound healing. Seeds are used to check all types of bleeding. Aqueous extract of whole aerial part is used as immunomodulator. Dry extract showed antibiotic activity even when formulated in mineral base.

FOR AUTHOR USE ONLY

Literature Review:

A WHO report stated that among 119 plants derived pharmaceuticals medicines, about 74% are employed in modern medicine in a way that directly related to their traditional uses as plant medicines by native traditional cultures. Many of the pharmaceutical firms are presently conducting extensive analysis and research on plant materials collected from the rainforests and other places for their potential medicinal value.

Antimicrobials are one of the most successful forms of chemotherapy and have been used to save the human population from the threat of infectious diseases. The emergence of microbial resistance to conventional antibiotics is a serious threat to the effectiveness of current antimicrobial therapy. To counteract antimicrobial resistance, discovery of novel antimicrobial agents to counteract the antibiotic-resistant strains is one of the major medical concerns of the 21st century. However, taking into consideration the current dynamics of development of drug resistance in the clinical isolates there is no assurance that new antimicrobials can overcome the problem. Thus the current requirement of antimicrobial therapy is to search for antimicrobial agents to which the pathogenic microbes cannot develop resistance easily. Nanomaterials can serve as a long-term solution to the ever-growing problem of antimicrobial resistance because they have shown antimicrobial effect against a wide range of drug-resistant infectious pathogens. The antimicrobial activity of nanomaterials is dependent on their ability to affect multiple biological pathways present in broad species of microbes. To develop microbial resistance to antimicrobial NPs, concurrent mutations have to occur, which makes NPs a prospective antimicrobial against which microbes may not develop resistance. Using NPs as carriers for conventional antibiotics has proved to enhance their antimicrobial activity against drug-resistant microbial strains and holds high

FOR AUTHOR USE ONLY

promise to overcome the problem of drug resistance. Thus the use of nanomaterials for treating microbial infections or for antimicrobial delivery could serve as a boon to overcome the current threat of antimicrobial resistance.

Antimicrobial chemotherapy plays a significant role in the management of infections. Many types of antimicrobial agents with different mechanisms of action, pharmacological properties and spectra of activity are available. Use of antimicrobial chemotherapy should be tailored to individual cases, with a good understanding of each drug's characteristics. It should be recognized that antimicrobial chemotherapy has potential negative effects including adverse ones. Moreover, use of antimicrobials provides intense selection pressure on the microbial populations to evolve resistance. Antibiotic resistance is currently one of the world's most pressing public health problems. It is of great concern that many clinicians still use antimicrobials inappropriately, with a poor understanding of the consequences. Proper use of these agents therefore remains an ethical duty for all healthcare professionals. The main classes of antimicrobial agents are disinfectants (non-selective agents, such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics (which are applied to living tissue and help reduce infection during surgery), and antibiotics (which destroy microorganisms within the body). The term "antibiotic" originally described only those formulations derived from living microorganisms but is now also applied to synthetic agents, such as sulfonamides or fluoroquinolones. Though the term used to be restricted to antibacterials (and is often used as a synonym for them by medical professionals and in medical literature), its context has broadened to include all antimicrobials. Antibacterial agents can be further subdivided into bactericidal agents, which kill bacteria, and bacteriostatic agents, which slow down or stall bacterial growth. In response, further advancements in antimicrobial technologies have resulted in

FOR AUTHOR USE ONLY

solutions that can go beyond simply inhibiting microbial growth. Instead, certain types of porous media have been developed to kill microbes on contact.

An antifungal medication, also known as an antimycotic medication, is a pharmaceutical fungicide or fungistatic used to treat and prevent mycosis such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and others. Survey suggests that, only 10 antifungal drugs are currently approved by the Food and Drug Administration (FDA) for the therapy of systemic fungal infections. As shown in table 1, these drugs belong to 3 principal classes: polyenes, pyrimidines, and azoles. Drugs that belong to other classes are also approved as topical antifungal drugs, but will not be considered further here.

FOR AUTHOR USE ONLY

Although conventional amphotericin B (Fungizone) remains the standard therapy for many invasive or life-threatening mycoses, this polyene drug is associated with significant toxicity, including infusion-related events, such as chills, fever, headache, nausea and vomiting, and dose-limiting nephrotoxicity . In addition, the clinical efficacy of amphotericin B in some settings (e.g., mold disease such as invasive aspergillosis in severely immunocompromised patients) is suboptimal.

Consequently, 3 new lipid formulations of amphotericin B (amphotericin B lipid complex, amphotericin B cholesteryl sulfate, and liposomal amphotericin B) have been developed and recently approved by the FDA. These lipid formulations offer several advantages over conventional amphotericin B, including increased daily dose of the parent drug (up to 10-fold), high tissue concentrations in the primary reticuloendothelial organs (lungs, liver, and spleen), decrease in infusion-associated side effects (especially liposomal amphotericin B), and marked decrease in nephrotoxicity. Although the therapeutic:toxic ratio of these compounds is

clearly improved, superiority in clinical efficacy has not been definitively established in head-to-head comparative trials, either a lipid formulation versus conventional amphotericin B or 1 lipid formulation versus another lipid formulation. Moreover, these lipid formulations of amphotericin B are considerably more expensive than conventional amphotericin B, ranging from 10- to 20-fold higher in cost per dose. In addition, the optimum daily or total dose of these lipid compounds has not been established.

The availability over the past 2 decades of the azole antifungal agents represents a major advance in the management of systemic fungal infections. Miconazole, the first azole drug to be approved and now recently withdrawn from the market, was available only as a highly toxic iv formulation; consequently, it was only rarely used. By contrast, the 3 oral azoles, ketoconazole, an imidazole, and, especially, itraconazole and fluconazole (both triazoles), have become frequently used therapeutic alternatives to amphotericin B . The relative broad spectrum of activity of the azoles against common fungal pathogens (e.g., *Candida* species, *Cryptococcus neoformans, Blastomyces dermatitidis, Histoplasma capsulatum, Coccidioides immitis, Paracoccidioides brasiliensis, Sporothrix schenckii*, and *Aspergillus* species [only itraconazole is active]), ease of administration, and limited toxicity are highly attractive features. Among the oral azoles, fluconazole (also available as an iv formulation) possesses the most desirable pharmacologic properties, including high bioavailability, high water solubility, low degree of protein binding, wide volume of distribution into body tissues and fluids, including cerebrospinal fluid, and urine, and long half-life. In addition, fluconazole and itraconazole are better tolerated and more effective than ketoconazole.One potential limitation of the azole antifungal drugs is the frequency of their interactions with coadministered drugs, which results in adverse clinical

FOR AUTHOR USE ONLY

consequences One type of azole-drug interaction may lead to decreased plasma concentration of the azole, related to either decreased absorption or increased metabolism of the azole. A second type of azole-drug interaction may lead to an unexpected toxicity of the coadministered drug, relating to the ability of the azoles to increase plasma concentrations of other drugs by altering hepatic metabolism via the cytochrome P-450 system. A second potential limitation of the azoles is the emergence of resistance of fungal organisms, especially *Candida* species, to fluconazole. Two situations illustrate this problem. First, several epidemiologic studies have correlated the increased frequency of non-*albicans Candida* species as causes of bloodstream infections with increased use of fluconazole for both prophylactic and therapeutic purposes. These non-*albicans Candida* isolates are often more resistant to fluconazole, compared with *C. albicans* isolates. Second, an increasing number of reports document clinical and/or microbiologic resistance to fluconazole in AIDS patients with oropharyngeal candidiasis, especially those who have a history of prolonged exposure to prior fluconazole and progressive immunosuppression. Available data indicate that the annual incidence of fluconazole resistant oropharyngeal candidiasis in AIDS patients is ~5%. These limitations of the azoles will become more problematic if fluconazole and other azoles continue to be used injudiciously (e.g., as prophylactic and empiric therapy in various patient groups without established indications).

FOR AUTHOR USE ONLY

Extensive resources have been devoted to develop reproducible and clinically relevant techniques for using *in vitro* susceptibility testing to predict *in vivo* response of mycoses to antifungal agents. As a result of multiple collaborative studies coordinated by the National Committee for Clinical Laboratory Studies, the M27-A broth dilution method has emerged as a reproducible tool for testing yeasts . By correlating results obtained by this method with outcome of therapy in both

mucosal and bloodstream *Candida* infections, data-driven interpretive breakpoints for fluconazole, itraconazole, and flucytosine have recently been proposed . On the other hand, reliable identification of amphotericin B-resistant isolates of *Candida* has proven technically difficult. Although recent results suggest that modifications of the underlying techniques of M27-A may produce clinically relevant results, convincing interpretive breakpoints have not yet been proposed. Likewise, meaningful determination of the susceptibility of *Cryptococcus neoformans* to any antifungal agent has proven technically difficult, and convincing interpretive breakpoints using the M27-A or any other method have not been proposed. Susceptibility testing methods for molds are still in the early stages of development. Based on these data, it is now reasonable to propose the use of antifungal susceptibility testing under certain scenarios. First, testing susceptibility of invasive (bloodstream or other sterile site) isolates of *Candida* against fluconazole should be performed on *C. albicans* isolates from patients with persistent candidemia or progressive disseminated candidiasis, despite fluconazole therapy, and on non-*albicans Candida* isolates (e.g., *C. glabrata, C. krusei*, or *C parapsilosis*) from patients with candidemia or invasive disease. Second, periodic testing of sterile site isolates of *Candida* may be useful to establish a local antibiogram that is helpful during selection of empirical therapy. Finally, susceptibility testing of mucosal *Candida* isolates from patients who have failed conventional therapy may be used to assist in determining the cause of the therapeutic failure.

FOR AUTHOR USE ONLY

The research is based on the antimicrobial and antifungal activity of Tridax procumbens linn.

Origin and Distribution:

Tridax procumbens Linn. is native of tropical America and naturalized in tropical Africa, Asia, Australia and India. This wild herb is distributed throughout India. Coat buttons are found on roadsides, waste grounds, railroads, dykes, riverbanks, meadows, and dunes. Its widespread distribution and importance as a weed are because of its spreading stems and plentiful seed production.

Description:

Tridax procumbens Linn. usually called as 'Ghamra' and in English popularly referred as 'coat buttons'. It is extensively utilized in an Ayurvedic system of medicine for varied ailments and is dispensed as "Bhringraj" by some of the practitioners of Ayurveda which is well-known medicine for liver disorders[1]

Chemical constituents:

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 GCMS The ethyl acetate soluble part of hexane extract yielded a new bithiophene named tri-bisbithiophene along with four terpenoids: taraxasteryl acetate, betaamyranone, lupeol and oleanolic acid . A new flavonoid (Procumbenetin) isolated from arial part of *T. procumbens* has been characterized as 3, 6-dimethoxy-5, 7, 2', 3', 4'-pentahydroxy flavones, 7- O-beta-3-glucopyranoside. Eight new compounds, isolated from *Tridax procumbens*, have been characterized as methyl 14- oxooctadecanoate, methyl 14-oxononacosanoate, 3- methylnonadecylbenzene, heptacosanyl cyclohexane caprylate, 1(2,2-dimethyl-3- hydroxypropyl)-2-isobutyl phthalate, 12-hydroxytetracosan-15-one, 32-methyl-30-oxotetratriacont-31-en-1-ol and 30-methyl-28- oxodotriacont-29-en-1-oic acid by spectral data and chemical studies. Nine known compounds isolated for the first time from the plant, were identif ied as dotriacontanol, β-amyrone, Δ12-

FOR AUTHOR USE ONLY

dehydrolupen-3-one, β- amyrin, lupeol, fucosterol, 9-oxoheptadecane, 10-oxononadecane and sitosterol.

The components of chemical present in plant are the oleanolic acid, fumeric acid, fl-sitosterol and tannin. In leaf extract contains alkaloids, carotenoids, flavonoids (catechins and flavones), saponins and tannins. Calcium, magnesium, potassium, sodium and selenium are the composition of minerals present in leaves. Luteolin, glucoluteolin, quercetin and isoquercetin in flowers. Leaf contains crude proteins – 26 percentage, crude fiber - 17 percentage soluble carbohydrates – 39 percentage calcium oxide - 5percentage. It also contains T. procumbens, including: saturated and unsaturated fatty acids, terpenoids, flavonoids, lipids, polysaccharides, such as β-sitosterol, puerarine, dexamethasone, esculetin, oleanolic acid, lupeol, quercetin, isoquercetin, fumaric acid, centaureidin, and luteoline .

FOR AUTHOR USE ONLY

Morphological Structure

Leaves

Leaves are irregularly toothed and generally arrow head shaped. They are simple, ovate, opposite, exstipulate, lanceolate and they are 3 - 7 cm. Wedge shaped base leaf, shortly petioled, hairy on both surfaces.

Fig 1 : Leaves of Tridax Procumbens

Stem

The plant stem is ascending 30-50cm height, branched, sparsely hairy, rooting at nodes.

Flowers

The plant flowers are looking like daisy .The flower is tubular, yellow centered white or yellow flowers with three-toothed ray florets. Inflorescence capitulum. It has two types of flower: ray florets and disc florets with basal palcentation. Sometimes the flowers are 3 lobed with long, penduncled heads. Achenes black narrowly conical, 2.0-2.5 mm long with feathery pappus. Flowering- Fruiting throughout the year.

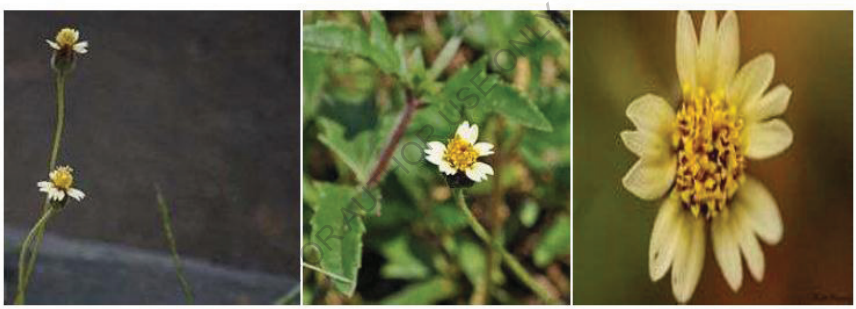


Fig 2: Flowers of Tridax Procumbens

Fruit

Fruit is a hard achene covered with stiff hairs and having a feathery. At one end It has plume like white pappus. The plant is invasive in part because it produces so many achenes and each achene can catch the wind in its pappus and be carried some distance.

Calyx

It is represented by scales or reduced to pappus.

Seed

The plant Seeds have pendulous embryo, endosperm is absent.

Fig 3: Seeds of Tridax Procumbens

FOR AUTHOR USE

Botanical Description

Kingdom **-** Plantae

Sub-kingdom **-** Tracheobionta

Division - Magnoliophyta

Class - Magnoliopsida

Sub-class - Asteridae

Order - Asterales

Family - Asteraceae

Genus - *Tridax*

Species - *procumbens*

Vernacular Names

English - Coat Buttons and *Tridax* Daisy,

Hindi - Ghamra,

Sanskrit - Jayanti Veda,

Marathi - Dagadi Pala,

Telugu - Gaddi Chemanthi,

Tamil - Thata poodu,

Malayalam - Chiravanak,

Spanish - Cadillp Chisaca,

French - Herbe Caille,

Chinese – Kotobukigiku

Traditional Uses:

Traditional and complementary medicine is being increasingly recognized as an integrative approach to health care in many countries. The use of plants for medicinal purposes may date back to the Middle Paleolithic age, approximately 60,000 years ago. T. procumbens is found throughout the world and it has been used to treat anemia, colds, inflammation, and hepatopathies in Central America. In Guatemala, T. procumbens is used as an antibacterial, antifungal, and antiviral treatment as well as for vaginitis, stomach pain, diarrhea, mucosal inflammations, and skin infections. The leaf juice is used to treat wounds and stop bleeding. A study done in Chiquimula, Guatemala, showed that lactating pregnant women suffering from anemia could reduce their symptoms by using Tridax. This species is also used in the treatment of gastrointestinal and respiratory infections, high blood pressure, and diabetes. In Guatemala, the entire plant is used for the treatment of protozoal, including malaria, leishmaniasis and dysentery. Aqueous extracts of T. procumbens have strong anti-plasmodial activity against chloroquine-resistant P. falciparum parasites. It has activity against Trypanosoma brucei, antibacterial and wound-healing properties.

FOR AUTHOR USE ONLY

Medicinal properties of different extracts of Tridax procumbens recorded in various literatures:

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 phytoconstituents 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.

FOR AUTHOR USE ONLY

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.

FOR AUTHOR USE ONLY

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 anti-dermatophytic 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. Helminthosporium oryzae, 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.

FOR AUTHOR USE ONLY

Anti-cancerous activity:

The in vitro anticancer activity of essential oil obtained from the leaves of T. procumbens was evaluated for MCF-7 cell line 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 transport 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 strandard 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.

FOR AUTHOR USE ONLY

Anti-obesity Activity:

In a research investigation, the animal receiving treatment with the plant decoctions showed a significant reduction in total cholesterol, triglycerides, total

protein, free fatty acids and elevation of high-density lipoprotein cholesterol levels.

Analgesic Activity:

Lyophilized decoctions of the plant were found to be potential analgesic.

Hypotensive Activity:

The cardiovascular effect was obtained from the water decoctions of leaves were investigated on anesthetized animals. The water decoctions can cause significant dose-dependent decreases in the mean arterial blood pressure. The higher dose leads to a significant reduction in heart rate whereas lower dose did not cause any changes in the same.

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.

FOR AUTHOR USE ONLY

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 caluculogenesis 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.

Cardiovascular effects

As per the survey, cardiovascular effects of aqueous extract from leaf of *T. procumbens* were investigated on anaesthetized Sprague-Dawley rat. The IV administration of 3, 6, and 9 mg/kg of aqueous extract caused significant decrease in mean arterial blood pressure in a dose related manner. Higher doses of drug also cause significant reduction in heart rate. The hypotensive and Bradycardiac effects were immediate. The hypotensive effect was inhibited by pretreatment of animal with atropine sulphate (1 mg/kg). The mechanism of action is possibly through activation of muscarinic cholinergic.

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 from 10 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 extrent than WPE. The prohealing 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.

FOR AUTHOR USE ONLY

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. A part 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.

FOR AUTHOR USE ONLY

Proteinase inhibitory activity:

Neutrophils are known to be a rich source of proteinase and are localized at lysozyme. It was previously reported that proteinases of leukocytes play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors 45.

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.

FOR AUTHOR USE ONLY

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 gastrointestinal 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 (IC50) at 3 µg/ml, showing its anti-leishmanial activity.

Anti-arthritic activity:

This study was conducted to assess the anti-arthritic activity of whole plant ethanolic extract of *Tridax procumbens* using Freundthe's Complete Adjuvant (FCA) model. Here arthritis was induced using FCA, and the anti-arthritic effect of the ethanolic extract of *Tridax procumbens* was evaluated at doses of 250 and 500 mg/kg and the effects were compared with indomethacin (10 mg/kg). At the end of the investigation, the liver enzyme levels were determined and a radiological examination was carried out. The study implies that *Tridax procumbens* at a dose of 250 and 500 mg/kg significantly inhibited FCA-induced arthritis in the rats.

FOR AUTHOR USE ONLY

Antiprotozoan activity

Tridax procumbens L. extracts were screened for antitrypanosomal properties in mice infected with Trypanosoma bruci and the report shown that insufficient antitrypanosomal activity.

Vasorelaxant effects:

The present study was designed to investigate the role of calcium in the vasorelaxant effect of this extract. Dose-response studies with noradrenaline (NA), potassium chloride and calcium chloride were carried out in rat aortic rings with and without the extract in physiological salt solution (PSS). Also, the role of intracellular calcium mobilization was noticed by measuring the phasic response to

NA in Ca^{2+}-free N, N-ethylene glycol tetra acetic acid (EGTA). The results of this investigation suggest that the vasorelaxant effect of *Tridax procumbens* leaf extract may be due to non-specific, non-competitive inhibition of Ca^{2+} influx as well as by inhibition of Ca^{2+} mobilization from intracellular stores. This implies that the extract may have vasorelaxant agents that may have calcium antagonistic activity .

Antihyperlipidemic activity

Tridax procumbens leave extract significantly decreased the accumulation of lipid content. By the presence of antioxidant molecules in extract they are having the Antihyperlipidemic activity. This activity tested on HepG2 cells. When the cells are treated with 20 mg/ml of extracts of *Tridax procumbens* and 1mM of oleic acid, no lipid accumulation observed in HepG2 cells. The leaves extract of *Tridax procumbens* had significant effect to decrease lipid content in HepG2 cells when compared with the normal model control. Hepatic lipid accumulation and oxidative stress effects leads to non-alcoholic fatty liver disease (NAFLD). Thus, potential therapeutic uses of hydroethanolic extract of *Tridax procumbens* in the prevention and treatment of hyperlipidemia and related diseases.

FOR AUTHOR USE ONLY

Anti juvenile hormone activity

Topical application of fraction of petroleum ether extractof *T. procumbens* showed remarkable effect on metamorphosis of Dysdercus and were found to be notable in generating abnormalities in adults due to juvenile hormone activity. Of fifteen plants tested, five plant extracts showed anti-juvenile hormone like activity against laboratory colonized late fourth in star larvae and adult female mosquitoes. Petroleum ether extract of Eichhornia *crassipes* and acetone extracts of *Ageratum conyzoides, Cleome icosandra, Tagetes erectes* and *T. procumbens* showed growth inhibitory and juvenile hormone mimicking activity to the treated larvae of C. quinquefasciatus. Larval pupal intermediates, demalanised pupae, defective egg

rafits and adult with deformed flight muscles were few noticeable changes. Biting behavior was observed to be affected only in ageratum, cleome and *T. procumbens* extracts. Loss of fecundity was observed in the treated mosquitoes but no sterilant effects could be seen. Adults, obtained from larvae exposed to the plant extracts produced significantly shorter egg-rafits than in control.

Defluoridation activity

Natural fluoride present in drinking water. Water companies added fluoride as a protective agent for teeth. However, in some natural drinking water, fluoride levels may be above safe level considered by the World Health Organization. Recently by using natural products has been rediscovered by water supply Technologists with more scientific rigor. Recently in India researchers have developed a filter system-based on a medicinal herb. This can be very quickly and easily remove "fluoride" from drinking water. *Tridax procumbens* was tested in water for the extraction of toxic heavy metals. It is suggested that *T. procumbens* can be used as a biocarbon absorbent for fluoride. By loading up plant tissue with aluminum ions possible to make a safe biocarbon filter. This filter readily absorbs fluoride ions from water warmed to around 27 °C passing through the filter.

FOR AUTHOR USE ONLY

METHODOLOGY:

Collection and Authentication of Plant Material

Fresh whole plant (leaves, stems, roots and flowers) of *Tridax procumbens* were collected from R. K. Nagar, Kolhapur, Maharshtra, India premises and authenticated by Dr. Miss. K. R. Datar (Head of Department of botany) Deccan Education Society Willingdon college, Sangli, Maharashtra, India. After authentication, fresh plant was collected in bulk, washed under running tap water, dried under shade for a period of 7 days and then pulverized in mechanical grinder to obtain coarse powder. The dried powder was stored in airtight bottles.

Chemicals

Methanol, Dextrose, Peptone, Agar, Distilled water, Ampicillin, Amphotericin B, Barium chloride dehydrate, Sulphuric acid.

FOR AUTHOR USE ONLY

Fungal strains

The bacterial and fungal strains for the study were obtained from Govt. Medical college, (Microbiology and bacteriology department). The fungal strains and bacterial strains used in the study are Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Aspergillus flavus, Candida albicans.

Ethanolic extract of *Tridax procumbens*

The coarse powdered material (each 100 gm) was soaked in 95% ethanol (500ml) by Soxhelation technique for continuous 72 hours. The extract was evaporated to dryness until dry mass is obtained. The yield obtained was 0.483%. [6]

Assesment of phytochemical screening, antibacterial and antifungal activity:

1. Preparation of Inocula – From fungal cultured slants, several colonies were transferred to 5ml of sterile distilled water. It is mixed for some seconds to ensure homogenicity and further diluted to match the turbidity with 0.5 McFarland standard solution corresponding to (1×10^6 CFU/ml).

2. Preparation of samples – i) *Tridax procumbens linn* alcoholic sample solutions were prepared at 5mg/ml, 10mg/ml, 15mg/ml concentrations in alcohol ii) Amphotericin B was taken as standard antifungal drug to study antifungal activity and Ampicillin was taken as standard antibacterial drug to study antibacterial activity.

3. Disc diffusion method -

i) Sabouraud Dextrose agar is prepared as fungal media and sterilized.

ii) All glasswares, filter disc, petriplates, extract dilutions were sterilized in autoclave.

iii) In aseptic technique, using sterile swab a bacterial lawn is made on sterile petri plates from microbial inoculums suspension. Swab is made in one direction by rotating plate at 90º.

iv) Sterile filter discs of 6mm diameter were impregnated with about 0.1ml/disc of each extract dilution solution and placed on agar plate in aseptic condition.

v) Plates are incubated at 28^{oc}-30^{oc} for 2 days. Alcohol, Sterile distilled water are kept as control. After 2 days zone of inhibition was measured. In case of alcoholic dilutions of *Tridax procumbens linn* the zone of inhibition of alcohol is subtracted from control alcohol zone of inhibition.

FOR AUTHOR USE ONLY

4. Agar Well Diffusion Method: The antibacterial activity of Tridax procumbens was evaluated by using agar well diffusion method. Bacterial cultures are mixed in nutrient agar medium and poured in Petriplates. Wells or cups of 5mm size were made with sterile borer into agar plates containing the bacterial inoculums. 2mg of crude *Tridax procumbens linn* was completely dissolved in 2ml of Ethanol 95%. Antibacterial activity was measured at different concentrations of extract ranging from 5,10,15 mg/ml drugs *Tridax procumbens linn*. The zone of inhibition of alcohol is subtracted from control alcohol zone of inhibition. Ethanol 95% served as control and antibiotic Ampicillin served as standard.

Preliminary Phytochemical Screening

The phytoconstituents present extracts of stem and root of Tridax procumbens were analyzed qualitatively by using standard procedures.

Test for Alkaloids:

About 2 ml of extract was taken and added 2 ml of concentrated HCL and then Mayer's reagent was added drop wise. The formation of white precipitate indicates the presence of alkaloids.

Test for Flavonoids:

The extract of 0.1 ml was taken and made up to 5 ml with distilled water, after which 0.3 ml of sodium nitrate was added and incubated for 5 mins at room temperature and then added 3 ml of 10% aluminium chloride which is incubated for 6mins at room temperature. Finally, 2ml of sodium hydroxide (NaOH) was added. The formation of yellow color indicates the presence of flavonoids.

FOR AUTHOR USE ONLY

Test for Saponins:

About 2ml of filtrate was mixed with 1ml of distilled water and shaken vigorously for about 3 seconds and it was allowed to stand for few mins and then added 3 drops of olive oil and shaken vigorously. Formation of emulsion indicates the presence of saponins.

Test for Terpenoids

About 1ml of the extract and 2ml of chloroform was taken and followed by the addition of 5ml of concentrated H2SO4 along the sides of the test tubes. Formation of a reddish brown coloration in the interphase indicates the presence of terpenoids.

Test for Phenolic Compounds

To 1ml of extract, 1ml of Iron (III) chloride was added and mixed well. A deep blue green color was formed which indicates the presence of phenolic compounds.

Test for Triterpenoids

A 10 mg of extract was dissolved in 1ml of acetic anhydride and then added 2ml of concentrated H2SO4. Formation of reddish violet color indicates the presence of triterpenoids.

Test for Quinones

To 2ml of plant extract, 1ml of concentrated H2SO4 was added. Formation of red color indicates the presence of quinones.

Test for Steroids

To 10 mg of plant extract, 2ml of acetic anhydride and followed by 2ml of H2SO4 were added. Formation of violet or blue color indicates the presence of steroids.

FOR AUTHOR USE ONLY

Test for Tannins

To1ml of the extract added 0.1% of ferric chloride solution and observed brownish green or a blueblack coloration which indicates the presence of tannins.

Test for Glycosides

About 1ml of extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicates deoxysugar which confirms the presence of cardenolides. A violet-green ring appearing below the brown ring in the acetic acid layer indicates the presence of glycosides.

Test for Coumarins

The extract was dissolved in methanol and then added alcoholic NaOH. A yellow color appears which later disappears on addition of drops of concentrated HCl indicates the presence of coumarins.

FOR AUTHOR USE ONLY

Result :

Tridax procumbens (15 mg/ml) showed maximum zone of inhibition was against Gram positive bacteria Staphylococcus aureus (18mm) and minimum against Gram negative bacteria Escherichia coli (8mm). *Tridax procumbens* (15mg/ml) showed maximum antifungal activity towards Aspergillus flavus (12mm) and Candida albicans (4mm).

Sr. No.	**Extract Concentration**	**Zone of Inhibition in mm of diameter**		
		Staphylococcus aureus	Pseudomonas aeruginosa	Escherichia coli
	Tridax procumbens linn			
1.	5mg/ml	10 mm	7 mm	8 mm
2.	10mg/ml	12 mm	10 mm	12 mm
3.	15mg/ml	18 mm	16 mm	18 mm
4.	Ampicillin (15mg/ml)	18 mm	9 mm	9 mm

FOR AUTHOR USE ONLY

Table 1: Effect of *Tridax procumbens Linn* extract on growth of bacteria *in vitro*. Zone of inhibition (mm)

Sr. No.	**Extract**	**Zone of Inhibition in mm of diameter**	
	Concentration	**Aspergillus flavus**	**Candida albicans**
	Tridax procumbens linn		
1.	5mg/ml	8 mm	4 mm
2.	10mg/ml	10 mm	8 mm
3.	15mg/ml	12 mm	10 mm
4.	Amphotericin B (15mg/ml)	8 mm	14 mm

Table 2: Effect of *Tridax procumbens Linn* extract on Pathogenic fungi Zone of inhibition (mm)

Table 3: Phytochemical Evaluation of *Tridax procumbens linn*

CHEMICAL TESTS	RESULT	CHEMICAL TESTS	RESULT
Test For Carbohydrates A. Benedicts Test B. Fehling's Test C.Molisch's Test	 Positive Positive Positive	**Test For Tannins** A.5% Ferric chloride B. Acetic acid test C. Dil. $KMnO_4$ Test	 Positive Positive Positive
Test For Steroids Salkowaski test	 Positive	**Test For Flavonoids** A. Lead acetate test B. NaOH + Dil.acid	 Positive Positive
Test For Alkaloids Dragendroff's test Wagner's test Mayer's test	 Positive Positive Positive	**Test for Glycosides** Borntrager's test	 Positive
Test for Saponins	Positive	**Test for Terpenoids**	Positive
Test for Triterpenoids	Positive	**Test for Quinones**	Positive
Test for Coumarins	Positive	**Test for phenolic compounds**	Positive

FOR AUTHOR USE ONLY

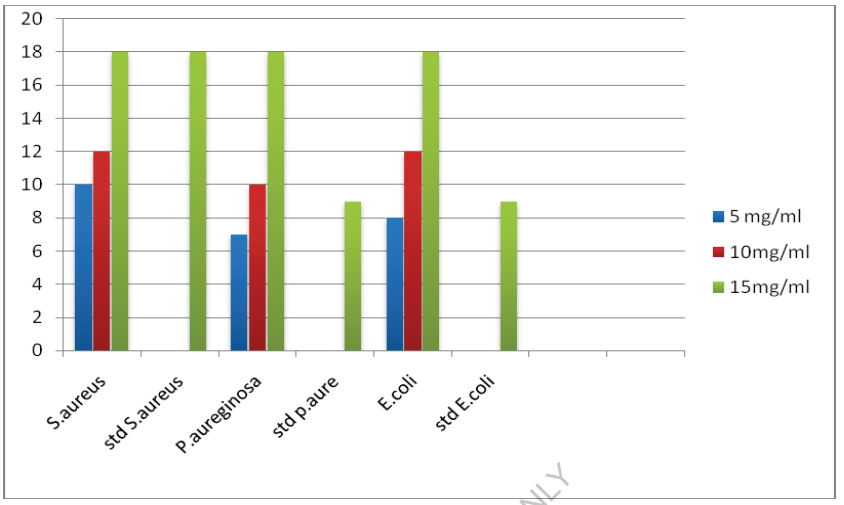
The phytochemical determination may be useful in the detection of the bioactive compounds and bionutrient may lead to drug discovery and development. Phytochemicals to provide health benefit for human in way of macronutrients and micronutrients. The chemical that protect plant cells from environmental hazard such as pollution, stress, drought, UV exposure and pathogenic effect are called phytochemicals. More than 4,000 phytochemicals have been investigated and catalogued. Alkaloids are used as anaesthetic agent and are found in medicinal plants. Terpenoids can have medicinal properties such as anticarcinogenic, antimalarial, antimicrobial and diuretic activities. Terpenoids are also very important in attracting useful mites and consume the herbivorous insects. Flavonoids are the most important plant pigments for flower coloration and as chemical messengers, physiological regulators, and cell cycle inhibitors. Coumarin

is a fragrant organic chemical compound in the benzopyrone chemical class, which is a colorless crystalline substance in its standard state. It is a natural substance found in many plants. Coumarins have shown some evidence of biological activity and have limited approval for few medical uses as pharmaceuticals, such as in the treatment of lymphedema.

The role of carbohydrates is to provide energy, as they are the body's main source of fuel for physical activity, brain function and operation of the organs. All the cells and tissues in our body need carbohydrates, and they are also important for intestinal health and waste elimination. Once in the body, carbohydrates are easily converted to fuel. Proteins also play a central role in biological processes. For example, proteins catalyze reactions in the system, transport molecules such as oxygen, keep healthy as part of the immune system and transmit messages from cell to cell. The water soluble vitamin C is functioning as enzyme cofactors and its deficiency leads to scurvy. Amongst the biological activities of flavonoids are actions against free radicals, free radical mediated cellular signaling, inflammation, allergies, platelet aggregation, microbes, ulcers, viruses, tumors and hepatotoxins . Phenolic acids are powerful antioxidants and have been reported to demonstrate antibacterial, antiviral, anti-carcinogenic, anti-inflammatory and vasodilatory actions. For centuries plants have been used for both nutritional and medicinal purposes. In conventional medicine is not cheap and a large population of the people depends on traditional medicine for their healthcare needs. Over the years, these herbal drugs have been shown to be effective. Many plants and their parts are used for the treatment of various diseases in different parts of the world, and are being screened for antimicrobial activities and the results obtained from these scientific studies have aided in the rationalization of medicinal use of these plants.

FOR AUTHOR USE ONLY

Antimicrobial activity:

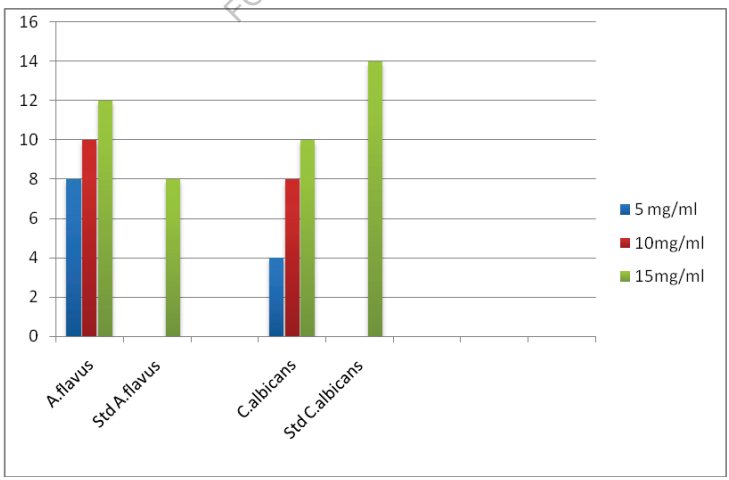


X axis: concentration in mg/ml Y axis: zone of inhibition in mm

Figure 4: Effect of *Tridax procumbens Linn* extract on Pathogenic bacteria

Zone of inhibition (mm)

FOR AUTHOR USE ONLY



X axis:concentration in mg/ml Y axis: zone of inhibition in mm

Figure 5: Effect of *Tridax procumbens Linn* extract on Pathogenic fungi Zone of inhibition (mm)

Antifungal activity:

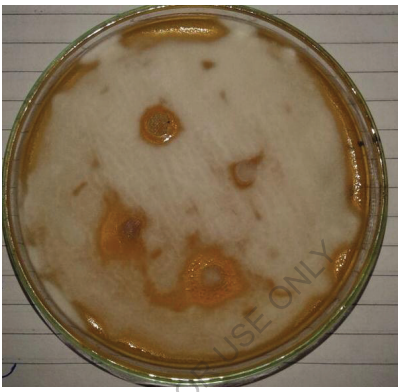


Figure 6: Effect of alcoholic extract of Tridax procumbens linn on Aspergillus Flavus

FOR AUTHOR USE ONLY

In the present study, active principles were extracted from T. procumbens and screened for various phytochemicals. The selected leaves showed the presence of phytochemicals such as, glycosides, flavonoids, saponins, steroids, alkaloids, carbohydrates, polyphenols and tannins. The methanol extract showed the presence of flavonoids, alkaloids, carbohydrates, polyphenols and tannins. The ethanol extract showed the presence of glycosides, flavonoids, carbohydrates, polyphenols and tannins. The term medicinal plant includes various types of plants used in herbalism. It is the use of plants for medicinal purposes, and the study of such uses. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used

drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments.

FOR AUTHOR USE ONLY

CONCLUSION

From the recent study it is concluded that, as dose of the *Tridax procumbens* increases the antimicrobial activity as well as antifungal activity increases. From the observations it clearly indicate that *Tridax procumbens* has potent antimicrobial activity as well as antifungal activity but it act by dose dependent manner. Also the study suggets, the ethanolic extracts of tridax procumbens L. inhibited the growth of all selected bacterial species but their effectiveness varied. The antibacterial and antifungal activity of the Tridax procumbens leaves and roots extract was done using agar well diffusion method. From this leaves and root extraction, leaves extraction has better inhibition properties of fungi and Bacteria. Leaves of tridax procumbans L were good hair growth promoters and have ability to prevent falling of hairs. This plant was also used as a good bioadsorbent for the removal of highly toxic heavy metals from industrial wastewater. Hence Tridax procumbens L. recommended for bioremediation.

FOR AUTHOR USE ONLY

DISCUSSION

The occurrence of antibacterial and antifungal substances in the higher plants in well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicines can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines of it can be the base for the development of a medicine, a natural blueprint for the development of a drug. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The results showed significant activity of *Tridax procumbens linn* and suggesting its use as natural antimicrobial agent. The result of present study indicated that ethanolic extract of Tridax *procumbens ,linn* shows potent antimicrobial and antifungal activity. Tridax procumbens L. was also reported for its anti-inflammatory and anti oxidant activity. leaves of tridax procumbans L were good hair growth promoters and has ability to prevent falling of hairs. This plant was also used as a good bioadsorbent for the removal of highly toxic heavy metals from industrial wastewater. Hence Tridax procumbens L. recommended for bioremediation. This plant was also used for bronchial catarrh, dysentery, diarrhea and for a remedy against conjunctivitis.

FOR AUTHOR USE ONLY

References:

1. Bushra Beegum NR and Ganga Devi T: Antibacterial activity of selected sea weeds from Kovalam South West coast of India. Asian Journal of Microbiology Biotech Env Sci 2003; 5(3): 319-322.
2. Mrs. Vishin Ashish Patil*, Mr. Sachin Annasaheb Nitave,Comparative Anthelmintic Activity and Phytochemical Evaluation of *Tridax procumbens Linn* Whole Plant Extract and *Piper nigrum Linn S*eed Extract on Indian Adult Earthworm , Int. J. Pharm. Sci. Rev. Res., 27(2), July – August 2014; 07, 54-57.
3. Ganju Kuldeep, Pathak AK, Pharmacognostic and Phytochemical Evaluation of *Tridax procumbens* Linn., Journal of Pharmacognosy and Phytochemistry, 1, 5, 2013, 42.
4. Jain Ankit, Amita Jain, Tridax procumbens: A weed with immense medicinal importance: a review, International Journal of Pharma and Bio Sciences, Vol 3,Issue 1, Jan – Mar 2012, 545.
5. R. R. Dhangar, S. M. Nikumbha*, M. M. Mane, H. P. Suryawanshi, S. P. Pawar, Investigation Of In-Vitro Anthelmentic Activity Of Tridax Procumbens, World Journal Of Pharmacy And Pharmaceutical Sciences ,Volume 4, Issue 06, 816-822.
6. http//e.m.wikipedia.org/Agar-diffusion-test
7. S.K. Shiva Rani, Neeti Saxena and Udaysree, Antimicrobial Activity of Black Pepper , Piper nigrum L., Global Journal of Pharmacology 7 (1): 87-90, 2013.
8. http//e.m.wikipedia.org/wiki/McFarland-standards
9. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. Medicinal plants in therapy. Bull World Health Organ. 1985; 63(6): 965.

FOR AUTHOR USE ONLY

10. Karimi A, Majlesi M, Rafieian-Kopaei M. Herbal versus synthetic drugs; beliefs and facts. J nephropharmacol. 2015; 4(1): 27.
11. Kethamakka SR, Deogade MS. Jayanti Veda (Tridax procumbens): Unnoticed medicinal plant by Ayurveda. J Indian Sys Medicine. 2014; 2(1): 6.
12. Udupa SL, Udupa AL, Kulkarni DR. Influence of Tridax procumbens on lysyl oxidase activity and wound healing. Planta Medica. 1991; 57(04): 325-327.
13. Pai C, Kulkarni U, Borde M, Murali S, Mrudula P, Deshmukh Y. Antibacterial activity of Tridax procumbens with special reference to nosocomial pathogens. Br J Pharm Res. 2011; 1(4): 164.
14. Appiah-Opong R, Nyarko AK, Dodoo D, Gyang FN, Koram KA, Ayisi NK. Antiplasmodial activity of extracts of Tridax procumbens and Phyllanthus amarus in in vitro Plasmodium falciparum culture systems. Ghana Med J. 2011; 45(4).
15. Hemalatha R. Anti-hepatotoxic and anti-oxidant defense potential of Tridax procumbens. Int J Green Pharm. 2008; 2(3): 164.
16. Mundada S, Shivhare R. Pharmacology of Tridax procumbens a weed. Int J Pharm Tech Res. 2010; 2(2): 1391-1394.
17. Poll E. Medicinal and aromatic plants of Guatemala and the need for their conservation. III WOCMAP Congress on Medicinal and Aromatic Plants. 2003; 167-170. 11. Raghavan TS, Vinkatasubban KR. Contribution to the cytology of Tridax procumbens Linn. Proceedings of the Indian Academy of Sciences. 1941; 13(2): 85-108.
18. Powell AM. Taxonomy of Tridax (Compositae). Brittonia. 1965; 47- 96. 13. Ananthakrishnan TN, Varatharajan R, Gopinathan K. Pollination in Wedelia

FOR AUTHOR USE ONLY

chinensis (Osbeck) Merr and Tridax procumbens L. (Compositae) by thrips (Thysanoptera: Insecta). Proc Indian Natl Sci Acad. 1981; 47(2): 159.

19. Chauhan BS, Johnson DE. Germination ecology of two troublesome Asteraceae species of rainfed rice: Siam weed (Chromolaena odorata) and coat buttons (Tridax procumbens). Weed Science. 2008; 56(4): 567-573.
20. Ravikumar V, Shivashangari KS, Devaki T. Hepatoprotective activity of Tridax procumbens against d-galactosamine/lipopolysaccharide-induced hepatitis in rats. J Ethnopharmacol. 2005; 101(1-3): 55-60.
21. Gaikwadi, Vadlamudi, Waghmaee, V.P., Maral, S.P., Ranteke, V.J. and Dhok, V.D. (2003). Phytochemical analysis of aqueous extract of few medicinal plants. Journal of Ethnopharrmacology. 2: 91-92.
22. Sahoo, M. and Chand P. K. (1998). In vitro multiplication of a medicinal herb Tridax procumbens (Mexican Daisy, coat button): influence of explanting season, growth regulator synergy, culture passage and planting substrate. Phytomorphology., 48: 195 – 206.
23. Meskin. and Mark, S. (2002) Phytochemicals in Nutrition and Health. CRC Press. p. 123.Global J. Pure Appl. Sci., 8b: 203- 208.
24. Suseela, L., Sarsvathy, A. and Brindha, P. (2002). Pharmacognostic studies on Tridax procumbens L. Journal of Phytological Research., 15: 141-142.
25. Tiwari, U., Rastogi, B., Singh, P., Saraf, D.K. and Vyas, S.P. (2004). Immunomodulatory effects of aqueous extract of Tridax procumbens in experimental animals. Journal of Ethanopharmacology., 9: 113-119.
26. Trease, G.E. and Evans, W.C. (1983). Textbook of Pharmacognosy. 12th Edition, Tindall and Co., London, 343-383.
27. Harborne, J.B. (1973). Phytochemical methods, Chapman and Hall, Ltd., London, pp. 49-188.

FOR AUTHOR USE ONLY

28. Wagner, H.M., Bladt, S. and Zgainski, E.M. (1984). Plant drug analysis. New York: springerverlag. p320.
29. Ranganna, S. (1976). Handbook of analysis of quality control for fruits and vegetable product. 2nd Edn. Tata McGraw Hill Publication Company, New Delhi, India. Pages: 545.
30. Roe, J. H. (1955). The Determination of sugar in blood and spinal fluid with anthrone reagent. J. Biol. Chem., 212(1): 335-43.
31. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Biochem., 193: 265 – 273. [13] Tabuti, J.R.S., lye, K.A. and Dhillion, S.S. (2003). Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. J. of Ethno pharmacology. 88:19-44.
32. Gibson, E.L., Wardel, J. and Watts, C.J. (1998). Fruit and vegetable consumption, nutrition knowledge and beliefs in mothers and children. Journal of Pharmacology and Phytochemistry. 31: 205 - 228.
33. Kappers, I.F., Aharoni, A., Van Herpen, T.W., Luckerhoff, L.L. and Dicke, M. (2005). Genetic engineering of terpeniod metabolism attracts bodyguard to Arabidopsis. Bioscience. 309: 2070- 2072.
34. Galeotti, F., Barile, E., Curir, P., Dolci, M. and Lanzotti, V. (2008). Flavonoids from carnation (Dianthus caryophyllus) and their antifungal activity. Phytochemistry Letters. 1: 44–48.
35. Farinola, N. and Piller, N. (2005). PharmacogenomicsIts role in re-establishing coumarin as treatment for lymphedema. Lymphatic Research and Biology. 3(2): 81–86.
36. Murray, R., Seifert, J. G., Eddy, D. E., Paul, G. L. and Halaby, G.A. (1989). Carbohydrate feeding and exercise: effect of beverage carbohydrate content. European Journal of Applied Physiology. 59: 152-158.

FOR AUTHOR USE ONLY

37. Dillard, C.J. and German, J.B. (2000). Phytochemicals: Nutraceuticals and Human Health. Journal of the Science of Food and Agriculture., 80: 1744-1756.
38. Mattila, P. and Hellstrom, J. (2006). Phenolic Acids in Potatoes, Vegetables, and Some of Their Products. Journal of Food Composition and Analysis., 20(3-4): 152-160.
39. Awe, S. and Omojalasola, P.F. (2003). Antibacterial screening of three medicinal plants used for diarrhea treatment in Ilorin, Nigeria. Nig. J. Pure and Appl. Sci., 1: 1375-1379.
40. Abo, K.A., Ogunleye, V.O. and Ashidi, J.S. (1999). Antimicrobial Potential of Spondias mombin, Croton zambesicus and Zygotritonia crocea. Phytother. Res., 13(6): 494-497.
41. Manisha N Trivedi, Archana Khemani, Urmila D Vachhani, Charmi P Shah, D D Santani, Pharmacognostic, phytochemical analysis and antimicrobial activity of Two piper species,International Journal Of Comprehensive Pharmacy, 2011, 2(7), 1-4.

FOR AUTHOR USE ONLY

FOR AUTHOR USE ONLY

Buy your books fast and straightforward online - at one of world's fastest growing online book stores! Environmentally sound due to Print-on-Demand technologies.

Buy your books online at
www.morebooks.shop

Kaufen Sie Ihre Bücher schnell und unkompliziert online – auf einer der am schnellsten wachsenden Buchhandelsplattformen weltweit! Dank Print-On-Demand umwelt- und ressourcenschonend produzi ert.

Bücher schneller online kaufen
www.morebooks.shop

KS OmniScriptum Publishing
Brivibas gatve 197
LV-1039 Riga, Latvia
Telefax: +371 686 204 55

info@omniscriptum.com
www.omniscriptum.com

OMNIScriptum

FOR AUTHOR USE ONLY

FOR AUTHOR USE ONLY