

 <p>ISSN NO. 2320-5407</p>	<p>Journal Homepage: - www.journalijar.com</p> <h2>INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)</h2> <p>Article DOI: 10.21474/IJAR01/4952 DOI URL: http://dx.doi.org/10.21474/IJAR01/4952</p>	 <p>INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR) ISSN 2320-5407 Journal homepage: http://www.journalijar.com Journal DOI: 10.21474/IJAR01</p>
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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND *IN-VITRO* ANTI-FUNGAL ACTIVITY OF “*TRIDAX PROCUMBENS L.*”

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Manuscript Info

Manuscript History

Received: 25 May 2017

Final Accepted: 27 June 2017

Published: July 2017

Key words:-

Anti-fungal activity, *Tridax procumbens L.*, Phytoconstituents, Other activities.

Abstract

In this study, our focus is to evaluate the role of bioactive molecule present in the essential oil of *Tridax procumbens L.* fresh leaves against bacterial and fungal species. The essential oil and flavonoids of *Tridax procumbens L.* has a number of properties that are beneficial to human health and well being. It may be used in the pharmaceutical and cosmetic industries for the development of new drug formulation for treating bacterial and fungal infections and also for treating inflammation related diseases in a cheaper rate with fewer side effects. The results of study have shown that the ethyl acetate and ethanolic extracts obtained from the *Tridax procumbens* were active against at least one of the opportunistic fungi the results of this study have shown that the leaf and whole plant extraction of *Tridax procumbens L.* Have great potential as antibacterial agents in the treatment of infectious organism. Further, detailed investigation of the active compounds of the plant for the extract mechanism of action will contribute greatly to the development new pharmaceuticals.

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Introduction:-

The demand on plant based therapeutics seems to increasing in both developing & developed countries due to the growing recognistic that they are natural products, being non narcotic, having no side effects, easily available at affordable prices and sometimes the only source of health care available to the poor. Plants have been used in developing countries as alternative treatments to cure diseases. Plant extracts are source of many potent and powerful drugs and many plant extracts and essential oils isolated have been shown to posses biological activity in vitro and in vivo, which justifies research on plant based medicine focused on the characterization of antimicrobial activity of these plants [1]. Thus the use of medicinal plants in disease treatment and prevention can also be seen as prehistoric and their present use can be supported by the traditional optimization of their application in disease control. Medicinal uses of plants range from administration of roots, barks, stems, leaves and seed to the use of extracts from the [2]. These plant extracts a sources of many potent and powerful drugs (3). Brazil and india are example countries that have a diverse flora and rich tradition in the use of medicinal plants both as antibacterial and antifungal applications. The antimicrobial activity of mrdicinal plants evaluated against enteric food borne pathogen (4). Antibacterial activities of some medicinal plants were observed earlier (5). Almost, all the cultivated crops are infected by one or more pathogens causing economic losses. The majority of the diseases are caused by fungi, bacteria and viruses. Apart from cultural methods are widely used to control the diseases caused by these pathogens.

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More use of bactericides and fungicides like organomercurial, carbamates etc. have posed serious problem to human and environmental health [6]. So research for natural bio-degradable source of bactericides and fungicides have always been quest for the researchers for control of bacterial and fungal disease of plants. Because of the present day public perception on pesticide contamination of foods especially the edible fruits, seeds, vegetables and oils, there is need for development of alternative economical and eco-friendly approaches for bacteria and fungal disease management. This could provide an alternative to the present day pollution problem of air, soil, water and residual effects of synthetic pesticides. with this view, the present investigation was undertaken to select plant extracts that could be effective in the development of new tools for the control of diseases caused by bacteria and fungi to the plants of economic importance. The major areas of drug from medicinal plants include disease conditions for which modern drugs are either unavailable or unsatisfactory (7). *Tridax procumbens* is a common annual weed. In west Africa sub region and tropical zones of world like india is known as “coat buttons”. Traditional medicine practitioners (TMPs) and tribal peoples of these areas use the leaves of plant as a remedy against several ailments ranging conjunctivitis, diarrhoea and dysentery to wound healing and related inflammatory conditions (8). Various studies have been carried out using plant extract of *tridax procumbens* in different solvents extraction such as total phenolics and antioxidant activity (9). Pharmacological evaluation (10),(11). Chemical profile (12), effect on lipopolysaccharide-induced hepatitis(13). Essential oil is so termed as they are believed to represent the very essence of odour and Flavour (14). There ia about three hundred essential oil in general use today by professional practitioners(15). Continuous damage has been encountered by viral, bacterial, parasitic and fungal contamination in our body. Essential oil plays a major role in protecting our body from this onslaught of pathogens, since immune system needs support and his essential oil can give the required endorsement(16). Esdevelopment of drugs. ntial oil is also found to have wide use in pharmaceutical industries in de In this study, our focus is to evaluate the role of bioactive molecule present in the essential Oil of *tridax procumbens* fresh leaves against bacterial and fungal species.



Plant profile:-

Scientific and biological classification of *tridax procumbens*

Classification	Scientific name	Biological name
Kingdom	Plantae	Plants
Sub kingdom	Tracheobionta	Vascular plants
Division	Spermatophyta	-----
Sub division	Mangnoliophyta	Flowering plants
Class	Mangoliopsida	Dicotyledons
Sub class	Asteridae	-----

Order	Asterales	-----
Family	Asteraceae	Aster family
Genus	Tridax l	Tridax
Species	Tridax procumbens l	Cocat buttons

Distribution:-

The plant is native of tropical America and neutralized in tropical asia, Africa, australia, and india. It is a wild herb distributed throughout india. It is also found along roadsides, waste grounds, dikes, riverbanks, meadows and dunes.

Propagation:-

It is not possible to micropropagate it through vegetative means for example cuttings. Propagation through seeds causes variations. Methods have been developed for its ex-situ conservation through micro propagation (17). Its wide distribution is due to spreading stems and abundant seed production (18).

Parts USED:-

Whole plant (leaf, stem and flower) is used to cure different ailments.

Morphological Features:-

Tridax procumbens L. is a small perrenial herb having short, blade like leaves. Transverse section studies of leaf along the midrib stem and roots were done.

Macroscopic of *Tridax procumbens L.*

PARTS	OBSERVATION
parts	Leaves
Arrangement	Opposite
size	3-7 cm long, 1-4 cm wide
shape	Lanceolate to ovate
colour	Green
Odour	Characteristic
Taste	Acrid
Appearance	Rough & scabrous
Margin	Irregularly toothed
Apex	Acute
base	Wedge- shaped
Petiole	Short
Texture	Short
Fracture	easy

Histological Studies Of *Tridax Procumbens L.*

Transverse section of the whole plant of *tridax procumbens* was studied. The section of leaf shows single layered upper epidermis consisting of polygonal tabular cells about 40-70 mewm by 15 to 30 mew m with asingle layer of cylindrical palisade cells about 18 to 30 mew m wide and 60 to 70 mew m long, spongy parenchyma 2-4 layered, cells polyhedral or isodiametric in shape.

Preliminary Phytochemical Screening Of Various Extracts Of The Whole Plant Of *Tridax Procumbens L.*

The coarsely powdered plant material was subjected to extraction by solvents with increasing polarity and the dried extracts were subjected to phytochemical studies using standard test procedures.

Phytochemical Screening:-

S.NO	CONSTITUENTS	ETHANOL	AQUEOUS	ETHYL ACETATE
1	alkaloids	(-)	(-)	(+)
2	Anthraquinone glycosides	(-)	(-)	(+)
3	flavonoids	(+)	(-)	(+)
4	Phenolic groups	(+)	(+)	(+)
5	saponins	(+)	(+)	(+)
6	tannins	(+)	(+)	(+)

7	steroids	(+)	(+)	(+)
8	carbohydrates	(+)	(+)	(+)

(+) indicates presence

(-) indicates absence

Chemical Constituents:-

A number of chemical constituents were reported from the plant *tridax procumbens* viz: Alkaloids, flavanoids, carotenoids, beta-sitosterol, n-hexane, fumaric acid, luteolin, quercetin, Oxoester, lauric acid, myristic, palmitic, arachidic, linoleic acid and tannin etc. Earlier Workers have reported presence of dexamethasone, luteolin, glucoluteolin, betasitosterol and Quercetin,(21), (22). linoleic acid was also reported in the aerial parts. A new flavanoid "procumbenetin isolated from aerial parts of plant, has been characterized as 3,6- dimethoxy-5, 7, 2', 3', 4'-pentahydroxyflavone 7- o-beta-glucopyranoside. Mineral composition of *T. Procumbens* reported from leaves is calcium, magnesium, potassium, sodium and selenium. It has been observed that *T. procumbens* can serve as a good source of plant protein and Potassium supplement, as well as being potential source of provitamin A (carotenoids) to the Population four new terpenoids along with bis-bithiphen were reported from *T. Procumbens*: taraxasteryl acetate, beta-amyrone, lupeol and oleonic acid. Two new Flavones, 8, 3'-dihydroxy-3,7,4'-trimethoxy-6-o-beta-D-glucopyranosal flavone(1) and 6,8,3'-trihydroxy-3,7,4'-trimethoxy flavone (2) were isolated from *tridax procumbens* linn., Together with the four known compounds puerarin(3), esculetin(4), olenolic acid (5) and Betulinic acid (6).

The plant yielded interesting compounds like luteolin, beta-amyrin, beta-amyrone, lupeol, tricontanol, fucosterol, campesterol, stigma sterol, besides arachidic acid, lauric acid, palmitic acid, flavones and glycosides.

Antifungal Activity Of The Three Extracts Of *Tridax Procumbens* :-.

The fungal was determined by measuring the diameter of zone of inhibition recorded. Ethyl acetate extract of *tridax procumbens* L. Was found to have maximum antifungal activity in comparison ethanolic and aqueous extracts. Ethyl acetate extract possessed potent antifungal activity against *Candida albicans* showing diameter of zone inhibition viz. 20mm while ethanolic extracts showed less antifungal activity against *Aspergillus flavus* and *Aspergillus niger* showing diameter of zone of inhibition viz, 12 mm and 18mm respectively. Ethyl acetate extract possessed potent antifungal activity against *Candida albicans* showing diameter of zone of inhibition viz., 12mm and 7 mm respectively. ethyl acetate extract possessed potent antifungal activity against *Candida albicans* showing diameter of zone of inhibition viz 12mm while ethanolic extract showed less fungal activity against *Aspergillus flavus* and *Aspergillus niger* showing diameter of zone of inhibition viz. 10 mm and 7 mm respectively.

Anti fungal activity of the three extracts of *tridax procumbens* L:-

S.no	Solvent extract	Organism	Diameter of zone inhibition (mm)	Mic(mg/ml)	Mfc(mg/ml)
1	EAE	<i>Candida albicans</i> ,	20	0.6	0.8
2	EE	-	12	0.7	0.9
3	AE	-	8	0.9	1.2
4	EAE	<i>Aspergillus flavus</i>	18	0.5	0.7
5	EE	-	12	0.7	1.0
6	AE	-	7	0.9	1.1
7	EAE	<i>A.niger</i>	12	0.6	0.8

Various Pharmacological Activities Of *Tridax Procumbens* L:-

Wound Healing:-

Leaf juice of *tridax procumbens* was shown to express wound contraction in experimental animals. *Tridax* antagonised anti-epithelialization and tensile strength depressing effect of dexamethasone (a known healing suppressant agent) without effecting anticontraction and antigranulation 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 this plant also promotes wound healing in both normal and immune compromised (steroid treated) rats, the plant increase not only lysyl oxidase but also, protein and nucleic acid content in the granulation tissue, probably as a result of increase in glycosaminoglycan content.

Hepatoprotective:-

The hepatoprotective activity of aerial parts of *tridax procumbens* was investigated against d-galactosamine\ lipopolysaccharide (d-GalN/LPS) included hepatitis in Rats. DGalN/LPS have been proposed to be hepatotoxic due to its stability to destruct liver cells. The multifocal necrosis produced by DGalN and the lesion of viral hepatitis in humans are similar. This amino sugar is known to selectively block the transcription and indirectly hepatic protein synthesis and as consequence of endotoxin toxicity, it contains fulminate hepatitis within 8 hour administration.

Anti-Diabetic:-

Masdhumeha another name of diabetes in which patient passes sweet urine And exhibits sweetness all over the body in the form of sugar, i.e. in sweat, mucous, urine, blood, etc. from ancient time various herbs were practically used for lowering of blood glucose level as such or in juices form. Aqueous and alcoholic extract of leaves of *tridax* showed a significant decrease in the blood glucose level in the model alloxan-induced diabetes in rats.

Antimicrobial Activity:-

Whole plant of *tridax* has reported for its antimicrobial activity on various species of bacteria. A whole plant is squeezed between the palms of hands to obtain juice. Fresh plant juice is applied twice a day for 3-4 days to cure cuts and wounds. The extract of whole plant of *tridax* showed antibacterial activity only against *Pseudomonas aeruginosa*. The disk diffusion method was used to test antimicrobial activity. Four strains of bacteria employed in test were two-gram positive *Bacillus subtilis*, *Staphylococcus aureus* and two gram negative *Escherichia coli* and *Pseudomonas aeruginosa*.

Anti-Cancerous Activity:-

The effect of cancer activity of traditionally plant *tridax procumbens* flower crude aqueous and acetone extract was tested on prostate epithelial cancerous cells PC3 was determined by measuring cell viability. Experiment consists of cleavage of the soluble yellow coloured tetrazolium salt MTT [3-(4,5-dimethyl -thiazole-2-yl)-2,5-diphenyl 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 then quantified spectrophotometrically at 570nm. The result of this analysis revealed the fact that flower crude extract has anticancer activity. Present study has been designed to determine the role of plant extracts of *tridax procumbens* L. both in aqueous and solvent extracts against pathogenic bacteria.

Discussion:-

The presence of antimicrobial substance in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytochemistry can be used for the treatment of diseases as done in case of unani and ayurvedic system of medicines if it can be the base for the development of a medicine, a natural blueprint for the development of a drug [17]. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but we found in this study the plant extracts by ethanol and methanol provided more consistent antifungal activity compared to those extracted by water. According to this study plant based antibacterial drugs have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobial agents. Naturally, the biologically active compounds whose activity can be enhanced in the presence of ethanol and methanol could have been produced. Number of active compounds responsible for antimicrobial activity. Essential oils are 'essences' and flavonoids are highly concentrated substances extracted from various parts of aromatic plants and trees. Unlike ordinary vegetable oils, such as corn and olive, plant essences are highly volatile and will evaporate if left in the open air. Essential oil is endowed with antiseptic, antibacterial, antibiotic, antidepressant, analgesic, decongestant and sedative properties. Moreover, due to their tiny molecular structure, essential oils applied to the skin can be absorbed into the bloodstream. They also reach the blood as a result of the aromatic molecules being inhaled. In the lungs, they pass through the tiny air sacs to the surrounding blood capillaries by the process of diffusion. The essential oils produced by the plants have been used traditionally for the respiratory tract infections and in the recent centuries the oils are used as ethical medicines for cold and microbial infections. By inhaling the vapours of the essential oil it shows the greater output of respiratory fluid and hence it is used in treating acute and chronic bronchitis, acute sinusitis and has an anti-inflammatory effect on trachea and reduced asthma. Earlier researchers report that alpha- and beta-pinenes are active against yeast and bacteria and this mechanism lies mainly in their capacity to induce toxic effects on the membrane structure and functions. Moreover, alpha-pinenes are used against mushrooms and yeasts (dermatophytes), especially on *Candida albicans* and other related species such as *Candida*

tropicalis, C.glabrat etc. the beta-pinenes also show antifungal properties, especially on candida spp. Studies have confirmed that the essential oil which is rich in alpha-pinene shows a potential anti-bacterial and anti-fungal activity. Major components of our plant *tridax procumbens* L. was employed using/mixing with aqueous for treating the antibacterial and other infections. In our study, we found that the *tridax procumbens* also contains the same compound as its major compound and the activity shown by the essential oil may also be due to the presence of these terpenes as its major compounds and other flavonoids. The anti fungal activity was determined by measuring the diameter of zone of inhibition recorded. Ethyl acetate extract of *tridax procumbens* L. was found to have maximum antifungal activity in comparison ethanolic and aqueous extracts. Ethyl acetate extract possessed potent antifungal against candida albicans showing diameter of zone of inhibition viz. 20mm while ethanolic extract showed less fungal activity against *asperigillus flavus* and *asperigillus niger* showing diameter of zone of inhibition viz. 12mm and 7 mm respectively. Ethyl acetate extract possessed potent antifungal activity candida albicans showing diameter of zone of inhibition viz. 12 mm while ethanolic extract showed less fungal activity against *asperigillus flavus* and *asperigillus niger* showing diameter of zone of inhibition viz. 10 mm and 7 mm respectively. The present study thus stated that, ethyl acetate extract of *tridax procumbens*.L was found to have maximum antifungal activity in comparison ethanolic and aqueous extracts and the plant is effective against fungal infections caused by candida albicans in comparison to *asperigillus niger*. Presence of chemical compounds viz, alkaloids, tannins, flavonoids and saponins of *tridax procumbens* L.

may inhibit the fungal growth, the present study provides the scientific information about the plant extract of *tridax procumbens* L. and supports the usage of this plant for curing fungal diseases by traditional healers. Further, phytochemical separation and immunological studies of this plant is in under progress.

Conclusion:-

The essential oil and flavanoids of *tridax procumbens* has a number of properties that are beneficial to human health and well being. It may be used in the pharmaceutical and cosmetic industries for the development of new drug formulation for treating bacterial and fungal infections and also for treating inflammation related diseases in a cheaper rate with fewer side effects. The results of this study have shown that the ethyl acetate and ethanolic extracts obtained from the *tridax procumbens* were active against atleast one of the opportunistic fungi the results of this study have shown that the leaf and whole plant extraction of *tridax procumbens* L. have great potential as antibacterial agent in the treatment of infectious organisms. Further, detailed investigation of the active compounds of the plant for the extract mechanism of action will contribute greatly to the development new pharmaceuticals

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