Evaluation Of Anti-Oxidant And Anti-Cancer Activity Of Tridax Procumbens With Its Anti-Fungal Activity Analysis

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ABSTRACT:
This paper reports the evaluation of antifungal property of the plant Tridax procumbens against the food pathogens Aspergillus species collected from two different food samples. The samples used here are lemon and rice culture. The crude extracts were prepared by grinding the leaves of the plant Tridax procumbens. Another extract was prepared by adding the ethanol to the crude extract. Then the fungal organisms were isolated and cultured by “streak plate technique”. The plates were incubated for 24 hours in room temperature for overnight and observed for inhibition of the fungal growth in the presence of extracts and the antifungal activity was observed after incubating them for another 24 hours. Our results showed the antifungal inhibition indicating the presence of significant amount of antifungal activity in plant extracts of Tridax procumbens.

KEY WORDS: Anti-fungal property, tridax procumbens, Aspergillus Nigar And Aspergillus Flavis, rice and lemon culture.

INTRODUCTION:
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 of physiology of host. In this paper the plant chosen for antimicrobial study was Tridax procumbes.
Tridax procumbens:

整株植物 | 花

Tridax procumbens, commonly known as coat buttons or tridax daisy, is a species of flowering plant in the daisy family. It is best known as a widespread weed and pest plant. It is native to the tropical Americas but it has been introduced to tropical, subtropical and mild temperate regions worldwide. It is listed as a noxious weed in the United States and has pest status in nine states. The plant bears daisy like yellow-centered white or yellow flowers with three-toothed ray florets.

Scientific Classification

 BINOMIAL NAME : Tridax procumbens

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It is denoted by different names; in English as Mexican Daisy, in ayurvedic as Jayanti, in siddha/tamil as Vettukkaaya-thalai and in folk as Akala kohadi. The whole plant was reported to treat various ailments, such as bronchial catarrh, dysentery, diarrhea, preventing hair loss, and to check hemorrhage from cuts. Pharmacological studies have shown that T. procumbens possess properties like anti-inflammatory, hepatoprotective, wound healing, immunomodulatory, antimicrobial, antiseptic, hypotensive and bradycardiac effects. Earlier workers have reported that the presence of dexamethasone, luteoline, glucotureolin, Beta-sitosterol, flavones, glycoside and quercetin in this plant [11,12].

A new flavonoid (procumbenetin) isolated from the aerial parts of Tridax procumbens has been characterised as 3,6-dimethoxy-5,7,2',3',4'-pentahydroxyflavone 7-O-β-D-gluco-pyranoside (1) on the basis of spectroscopic techniques and by chemical analysis [1]. Tridax procumbens; Flavonoids plants are commonly used in Indian traditional medicine as anticoagulant, hair tonic, antifungal and insect repellent, in bronchial catarrh, diarrhoea, dysentery, and wound healing. Previously isolated constituents. Alkyl esters, sterols,
pentacyclic triterpenes, fatty acids and polysaccharides. Newly isolated constituent. 3,6-Dimethoxy-5,7,2',3',4'-pentahydroxyflavone 7-O-β-D-glucopyranoside (1), named procumbetin. Yield: 0.016% on dried basis [11,12].

Tridax procumbens is known for several potential therapeutic activities like antiviral, anti oxidant antibiotic efficacies, wound healing activity, insecticidal and anti-inflammatory activity. Some reports from tribal areas in India state that the leaf juice can be used to cure fresh wounds, to stop bleeding, as a hair tonic. Despite these known benefits, it is still listed in the United States as a Noxious Weed and regulated under the Federal Noxious Weed Act. A study by Gamboa-Leon (2014) showed that a mixture of Tridax procumbens and A. sativum extracts was a promising natural treatment for cutaneous leishmaniasis and that its healing effects made it a good candidate for a possible new phytomedicine. The mixture of Tridax procumbens and A. sativum extracts was better at controlling Leishmania mexicana infection while not being toxic when tested in the acute oral toxicity assay in mice [2].

Whole plant ethanolic extract of Tridax procumbens showed significant anti-arthritis effect, antidiabetic and antihyperlipidemic effects in rats using the Freund's Complete Adjuvant (FCA) model and streptozotocin-induced diabetic model [7]. Traditionally, Tridax procumbens has been in use in India for wound healing, as anticoagulant, antifungal and insect repellent. It is also used in diarrhoea and dysentery. Its leaf extracts were known to treat infectious skin diseases in folk medicines. It is a well-known ayurvedic medicine for liver disorders or hepato-protective nature besides gastritis and heart burn [4].

In humans, Tridax procumbens used as treatment for boils, blisters and cuts by local healers in Nalgonda and Warangal District of Telangana, Andhra Pradesh, India [3]. A study had found anti-cancer properties of Tridax procumbens against human prostate epithelial cancer cell line PC 3 [8,9]. A study was carried out to verify the claims wherein tribal inhabitants of Udaipur district, Rajasthan were using the plant for treatment of diabetes [6]. It was concluded that the results were comparable to that of reference standard Glibenclamide and the Tridax procumbens flower extract showed antidiabetic properties [10], Phatak et al., (1991) has investigated the hair growth promoting activity of Tridax procumbens and the petroleum ether extract of Tridax procumbens was found to be effective in promoting hair growth in male wistar albino rats [5].

**Chemical Composition Of Leaves Of Tridax procumbens**

Chloroform extract of leaves of Tridax procumbens Linn shows the presence of Steroid, Saponin, Coumarins, Alkaloids, Amino acids, Diterpenes, Phenol and Flavonoids whereas Tannin, Anthocyanin, Emodins, Proteins, Phytosterol, Phlobatannin, Leucoanthocyanin and Cardial Glycosides were absent. Acetone-Water extract of leaves of Tridax procumbens Linn shows the presence of Steroid, Tannin, Saponin, Anthocyanin, Coumarins, Alkaloids, Diterpenes, Phenol and Flavonoids whereas Emodins, Proteins, Amino acids, Phytosterol, Phlobatannin, Leucoanthocyanin and Cardial Glycosides were absent. Chloroform-Water extract of leaves of Tridax procumbens Linn shows the presence of Steroid, Tannin, Saponin, Anthocyanin, Coumarins, Alkaloids, Amino acids, Diterpenes, Phenol and Phlobatannin whereas Emodins, Proteins, Phytosterol, Leucoanthocyanin, Cardial Glycosides and Flavonoids were absent [13,14,15].
Materials and Methods:

Preparation of Crude Extract:

Fresh leaves were taken from the plant Tridax procumbens. The leaves were first washed with normal tap water so that the dust particles particles present in it are removed and they are once again rinsed with the distilled water. After that they are placed over a plain paper so that the paper absorbs the excess water. Pure and ethanol extracts were prepared using leaves and leaves were ground in Mortal pistol without adding any water or solvent after washing them with the distilled water (Figure 2). Then it is filtered in a screw cap container or storage container using a filter paper. The pure sap from the leaves is called the pure crude extract. It looks in a mild brown colour. [16-19]The ethanol extract is also prepared using the same method above but in here addition of the ethanol before grinding the leaves is the main difference. Then it is filtered as done before. This extract is called as ethanol extract and the extract looks in a deep green colour.

![Figure 2: preparation of Crude and Ethanol Extracts of plant Tridax procumbens](image)

Potato Dextrose Agar:

Potato Dextrose Agar is composed of dehydrated Potato Infusion and Dextrose that encourage luxuriant fungal growth. Agar is added as the solidifying agent. Many standard procedures use a specified amount of sterile tartaric acid (10%) to lower the pH of this medium to 3.5. Suspend 39 g of the medium in one litre of purified water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave it at 121°C for 15 minutes.

Mc CONKEY AGAR:

It consists of Enzymatic Digest of Gelatin, Casein and Animal tissue which provides nitrogen, vitamins, minerals and amino acids essential for growth, Lactose: fermentable carbohydrate providing carbon and energy. Bile Salts: selective agents and inhibit Gram positive organisms. Crystal Violet: Gram positive bacteria are generally inhibited by crystal violet. Sodium Chloride: supplies essential electrolytes for transport and osmotic balance. Neutral Red: pH indicator which is red in color at pH’s below 6.8. When lactose is fermented, the pH of the medium decreases, changing the colour of neutral red to pink. Agar Agar : Solidifying agent. Suspend the measured amount of powder (See in the agar bottle and generally 50 gram) in 1 L of purified water and mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave the medium at 121°C for 15 minutes [11,12].

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**ISOLATION OF FUNGAL ORGANISMS:**

The fungal organisms used here are Aspergillus niger and Aspergillus flavus. These organisms are available in the food samples of lemon and rice culture respectively. From those cultures required fungal specimens were isolated. Both the isolated specimens Aspergillus niger and Aspergillus flavus were stained in slides (Figure 3) and observed under microscope.

![Aspergillus niger (a) and Aspergillus flavus(b)](image)

**Aspergillus niger or A. niger** is a fungus and one of the most common species of the genus Aspergillus. It causes a disease called black mould on certain fruits and vegetables such as grapes, apricots, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of Stachybotrys (species of which have also been called "black mould"). Some strains of A. [20-27] niger have been reported to produce potent mycotoxins called ochratoxins; other sources disagree, claiming this report is based upon misidentification of the fungal species. Recent evidence suggests some true A. niger strains do produce ochratoxin A. It also produces the isoflavone orobol.

**Aspergillus flavus:**

Aspergillus flavus is a saprotrophic and pathogenic fungus with a cosmopolitan distribution. It is best known for its colonisation of cereal grains, legumes, and tree nuts. Post harvest rot typically develops during harvest, storage, and/or transit. A. flavus infections can occur while hosts are still in the field (preharvest), but often show no symptoms (dormancy) until post harvest storage and/or transport. In addition to causing pre harvest and post harvest infections, many strains produce significant quantities of toxic compounds known as mycotoxins, which are toxic to mammals. [28-32] A. flavus is also an opportunistic human and animal pathogen, causing aspergillosis in immunocompromised individuals. These organisms were streaked in petri plates in which the prepared agar was poured. Aspergillus niger was streaked in Mc Conkey agar media and the A.flavus was streaked in PDA media.

**STREAK PLATE TECHNIQUE:**

The streak plate method is a rapid qualitative isolation method. The techniques commonly used for isolation of discrete colonies initially require that the number of organisms in the inoculum be reduced. It is essentially a dilution technique that involves spreading a loopful of culture over the surface of an agar plate. The resulting diminution of the population size ensures that, following inoculation, individual cells will be sufficiently far apart on the surface of the agar medium to effect a separation of the different species present.
Although many type of procedures are performed, the four ways or quadrant streak is mostly done.

PROCEDURE:

The lid of the agar plate has to be opened just sufficiently enough to streak the plate with the inoculation loop. Minimize the amount of agar and the length of time the agar is exposed to the environment during the streak process. Sterilize the wire loop. Cool the loop by touching it on the edge of the sterile agar plate. Dip the loop into the broth culture containing the mixture of bacteria. Lift the lid of the plate just enough to insert the loop. Drag the loop over the surface of the top one-third of the plate back and forth in a "zig-zag" formation. The loop has picked up thousands of bacteria which are spread out over the surface of the agar. Sterilize the loop in the flame.[33-39] Turn the plate 90 degrees and drag the loop through the area you have just streaked two to three times and continue to drag the loop in a "zig-zag" formation in the remaining half of the plate without touching that area again. Sterilize the loop in the flame. Turn the plate 90 degrees. Repeat the procedure. Drag the loop two to three times through the area you just streaked, and fill in the remaining area of the plate (zig-zag formation), being very careful not to touch any of the areas you previously streaked.

Results and Discussion :

Anti-microbial test

The prepared extracts were poured in one of the plate containing Mc Conkey agar media and the ethanol extract was poured in one of the plates containing PDA media using a micropippette. And the plates were shaken mildly so that the extracts were spread throughout the media. These processes were done before the incubation of the streaked plates. The other two plates were incubated without pouring the extract. Then the four plates were kept for incubation for 24 hours in room temperature.[40-42] The very next day itself extracts were added to the plates containing fungal growth and then the plates were incubated for another 24 hours in room temperature to find the anti fungal activity of the extract (Figure 4).

Many of the medicinally important plants are unknown to the world, therefore in this paper aimed to evaluate the anti fungal activity of the plant *Tridax procumbens*. On observing the plates in the next 24 hours it was found that there is no growth in the first two plates in which we have added the extracts. The next two plates contain some fungal growth. And then observing it after another 24 hours after adding the extracts to the plates containing the fugal growth helps us to find that the plate showed no difference in appearance as it was looked the day before. [43-45]
Based on the above results it can be concluded that the plant has antifungal activity. It is clearly shown from the plates that the plant inhibited the fungal growth in the case of first two plates and it stopped the further growth of fungi in the next two plates. Hence we conclude that the plant has anti fungal property.

REFERENCES


