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Bioautography assay of *Caesalpinia coriaria* (Jacq) wild, as antifungal agent.

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Abstract

Bioautography identification of *Caesalpinia coriaria* as antifungal compounds on the TLC plates was studied. An important development of drug discovery process over the last several decades has been in the use of biological assay systems. Based on this the ethanolic extract of *C.coriaria* were subjected for bioautography assay. The fractions were tested for their antifungal activity against *Mucor* organism. Active compounds alkaloids, flavonoids, terpenoids and cardiac glycosides from the fractions are transferred from stationary phase to the agar layer (which contains the microorganisms) by a different process. After incubation the plate is sprayed with a tetrazolium salt (eg. MTT) which is converted to a formazan dye by the microorganism. Inhibition zones are observed as clear spots against a purple background with this knowledge, it is clear that in the present study the active compound fractions 12 at R_f showed significant inhibition towards the growth of pathogenic microorganisms. It showed the presence of bioactive compounds responsible as antifungal properties of ethanolic partially purified extract. However, this finding provides an insight into the usage of this plant in traditional treatment of foot infections, subcutaneous parasitic infection, intestinal parasitism, venereal diseases and other diseases associated with bacterial and fungal infections.

Keywords: *Caesalpinia coriaria*, bioautography, *Mucor*, tetrazolium salt, MTT.

Introduction

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in history of all civilizations. Man in the pre-historic era was probably not aware about the health hazards associated with irrational therapy. With the onset of research in medicine, it was concluded that plants contain active principles, which are responsible, for curative action of the herbs, Ancient knowledge coupled with scientific principles can come to the forefront and provide us with powerful remedies to eradicate the diseases (Sarah H. Bates, 2000).

The phytochemical isolated are then screened for different typed of different types of biological activity.

Cytotoxicity via the brain shrimp test is studied in order to several new anticancer compounds. Alternation crude plant extracts can be first assay for particular activities and the active fractions then analyzed phytochemically. A variety of bioassays are now available for the phytochemical to use in such work (Cuendet M, 1997).The medicinal value of plant is due to the presence of certain secondary metabolites such as glycosides, resins, gums, alkaloids, volatile oil and tannins (P.K. wong, 2012). Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases (J. Gonzalez, 1980). In the recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal

plants used in various traditional, complementary and alternate systems of treatment of human diseases (Sher A, 2009). Recently, some higher plant products have attracted the attention of microbiologists to search for some phytochemicals for their exploitation as antimicrobials, such plant products would be biodegradable and safe to human health (Kumar A, 2008). Crude extracts of some well known medicinal plants are used to control some of the plants pathogens (Kubo M, 1981).

Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Ibahim MB, Ogundipe O, Akinbiya O, 1997, 1998). The effects of plant extracts on bacteria have been studied part of the world (Reddy PS, 2001). Much work has been done on ethno medicinal plants in India (Mohana *et al.*, 2006; Negi *et al.*, 1993). Interest in a large number of traditional natural products has increased (Tarik *et al.*, 2012). The fungi are major disease causing agents on plants and can lose up to 90% agricultural yield. Plants contain hundreds or thousands of metabolites. Medicinal and aromatic plants a gift of nature are being used against various infections and diseases in the world since past history (Samson *et al.*, 2001).

Materials and Methods

Collection of plant materials

Leaves of *Caesalpinia coriaria* free from diseases were collected from Captain Srinivasa Drug Research Institute, Anna Nagar, Chennai.

Culture media

The media used for antifungal test was Sabouraud's dextrose agar. 4 gram of SDA was weighed and dissolved in 75 ml of distilled water.

Inoculum

The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6h and the suspensions were checked to provide approximately 10^5 CFU/ml.

Fungal strains used

The clinical fungal test organisms used for study are *Aspergillus niger* NCIM 545, *Rhizopus oryzae* NCIM 902, *Mucor* sp. NCIM 881 and *Penicillium* sp.

Extract preparation:

2 kg of leaves of *Caesalpinia coriaria* were shade dried separately, coarsely powdered and soaked in ethanol in aspirator bottles and exhaustively extracted at room temperature for 72 hours. The ethanol extract was completely dried from solvent under reduced pressure using high vacuum conditions. The collected extracts were taken up for further investigations.

Pour plate method

Liquefied agar as SDA (4 gm in 75 ml of distilled water) was boiled to melt the agar. Agar at that temperature would kill the fungi when introduced so the agar was cooled to 60 Celsius and held in the water bath to maintain the temperature. This would not kill the fungi when they are introduced to the liquid agar and will reduce the amount of consideration that will collect on the lid of Petri dish. The agar will solidify at 42 Celsius then the drop of culture was aseptically transferred to it. Mix the tube by rolling the tube between your hands. Pour the inoculated liquid into the sterile Petri dish that was labeled now move the dish to cover the bottom of the disc with agar. Allow the agar to solidify so this would take an hour. Inoculate the plate at 37 Celsius or 24 hours to 48 hours. 50 micro gram of inoculated *Mucor* sp was poured on the agar media on conical flask mix it well and poured on petri dish and inoculated at 37 Celsius. Colonies were obtained from surface of Petri dish.

Identification of the antifungal compounds on the plate through bioautography

SDA overlay TLC bioautography method (Sawaya ACHF., 2004) was used to detect the effective antifungal compounds from *Caesalpinia coriaria*. The inoculum of fungi was distributed over the already prepared TLC plate. After solidification, the TLC-bioautographic plate was incubated at 37°C for 24 hours. The bioautogram developed was sprayed with 1% aqueous solution of methyltetrazolium (MTT) and incubated at 37°C for 4 hours. Inhibition zones indicated the presence of active compounds.

Growth inhibition areas were identified by the Rf of the related spots on the reference TLC plate. Preparative TLC plates with a thickness of 1mm were prepared using same stationary and mobile phase as above with the objective of isolating the components of plant extracts that inhibited the growth of fungal isolates.

Results and Discussion

Bioautographic identification of antifungal compounds on TLC plates

TLC bioautography SDA agar overlay method is considered as one of the most efficient methods of the detection of antifungal compounds. It involves the

transfer of the active compounds from the stationary phase into the SDA agar layer through a diffusion process. Eventually, the bioautography of the TLC plates of the isolated fractions of *Caesalpinia coriaria* showed an area that inhibited the growth of organism over the region containing the active compound of fractions 6, 7, 8, 10, 11 and 12 was at Rf.



Figure 1 : TLC- Bioautography of *Caesalpinia coriaria* Fractions Tested against Pathogenic Fungi before Staining with Methyl Tetrazolium

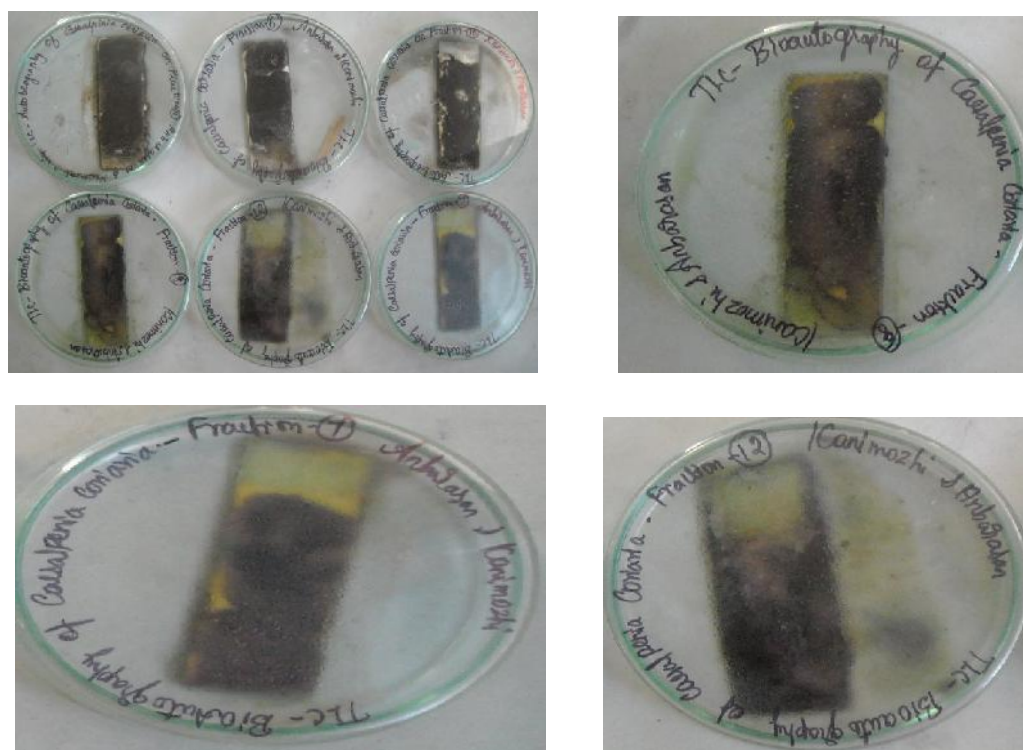


Figure 2 :TLC- Bioautography of *Caesalpinia coriaria* Fractions Tested against Pathogenic Fungi after Staining with Methyl Tetrazolium

Partial characterization of phytoconstituents from the leaves of *Caesalpinia coriaria*

TLC is the simplest and cheapest method of identify plant constituents because the method is easy to run, reproducible and requires little equipment (Newman *et al.*, 2000). It is also easy to monitor the progress of

a reaction, detect the compounds present in the phytochemical extract and to determine the purity of the compound. Based on this use, the different phytochemical extracts alkaloids, terpenoids, flavonoids and glycosides of *Caesalpinia coriaria* resolved the various compounds at varying levels of

Rf values. It was also possessed the maximum number of compounds in the isolated fractions. Active compounds alkaloids, flavonoids, terpenoids and cardiac glycosides from the fractions are transferred from stationary phase to the agar layer (which contains the microorganisms) by a different process. After incubation the plate is sprayed with a tetrazolium salt (eg. MTT) which is converted to a formazan dye by the microorganism. Inhibition zones are observed as

clear spots against a purple background with this knowledge, it is clear that in the present study the active compound fractions 12 at Rf showed significant inhibition towards the growth of pathogenic microorganisms. There are many reports on plants having medicinal values and the compounds responsible for antimicrobial the activity (Ebna 1991, Aya 2011).



Figure 3 Bioautography plate compared with TLC Plate (Alkaloids)



Figure 4 Bioautography plate compared with TLC Plate (Terpenoids)

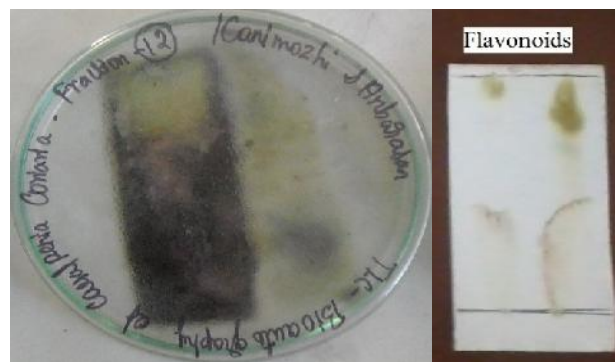


Figure 5 Bioautography plate compared with TLC Plate (Flavonoids)

Summary and Conclusion

Many of the modern pharmaceuticals used today for various ailments were based on plants and plant-based medicaments (Abraham, 1981). The first step towards the goal of ethnopharmacological study was the invitro antifungal activity assay. The antibacterial, antioxidant, cytotoxic potential of *Caesalpinia coriaria*. Some of those observations have helped in identification of the active principle responsible for such activities. However, the actual exploitation of *Caesalpinia coriaria* for antifungal property was not reported.

The research for naturally occurring materials with biological activity and use of occurring antifungal substance in plant chemotherapy is gaining more importance (Entomol, 1990). In the present study revealed that the ethanol extract of *C. coriaria* showed the positive activity against *Mucor* sp. The study indicated that the inhibitory effect of plant extract depend on the polarity of the solvent and also due to the presence of aqueous, acetone, ethyl acetate and ethanol and quantified. This study provides new scientific information on antifungal activity of *C. coriaria* against *Mucor* sp. The research is still progress on bioactivity guided, isolation and structural elucidation of the bioactive compounds responsible for the observed antifungal activity.

In the present study, the ethanolic leaf extract of *Caesalpinia coriaria* was analysed for their inhibitory activity against *Aspergillus niger* NCIM 545, *Rhizopus oryzae* NCIM 902, *Mucor* sp. NCIM 881 and *Penicillium* sp. The extracts of the plant used showed prominent antifungal activity against *Mucor* sp. The ethanolic extract provides antifungal activity than the aqueous, acetone and ethyl acetate extracts.

The research for naturally occurring materials with biological activity and use of occurring antifungal substance in plant chemotherapy is gaining more importance (Entomol, 1990).

Considering the wide usage of these plants as source for antifungal activity a systematic investigation was undertaken to screen the potentiality of *Caesalpinia coriaria* as antifungal. Initially, the bioactive compounds responsible for the antifungal activity. This plant possessed among the Phytochemicals identified, fraction 12 was found to have antifungal activity against *Mucor* sp.


Thus the use of these plants in the treatment of pathogenic diseases associated with the infection of these pathogens is validated, scientifically supported by the results obtained in this work.

The phytochemical analysis revealed the presence of important secondary metabolite (alkaloids, saponins, tannins, steroid, flavonoids and cardiac glycosides) thus, indicating the therapeutic potentials of *C. coriaria*. It showed the presence of bioactive compounds as well as the antifungal properties of ethanolic partially purified extract. However, this finding provides an insight into the usage of this plant in traditional treatment of foot infections, subcutaneous parasitic infection, intestinal parasitism, venereal diseases and other diseases associated with bacterial and fungal infections.

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