AN ANALYTICAL STUDY OF ANTIFUNGAL ACTIVITY OF CRUDE EXTRACTS AND ACTIVE CONSTITUENTS OF INDIAN HERBAL PLANTS

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Abstract

In the light of late issues of numerous drug resistance and intractable microbial diseases, the need to discover new substances with antimicrobial properties winds up plainly correlated. The present work encompasses the screening of aqueous and methanol/ethanol extracts of 108 plant species randomly collected, for their antibacterial property against a wide array of microorganisms. Further, the most active plant extracts indicating best antibacterial activity were screened for antifungal activity. The most encouraging plant extract was chosen for facilitate phytochemical and pharmacological activities. This is in pursuance of the endeavors to search for drugs from plants and the verification of the logical basis of some known practices in traditional medicine.

1. INTRODUCTION

Infectious diseases caused by bacteria, fungi, viruses and parasites are as yet a major threat to public health, regardless of the gigantic advance in human medicine. Their impact is particularly large in creating nations because of the relative unavailability of medicines and the rise of widespread various drug resistances because of indiscriminate utilization of commercial antimicrobial drugs normally utilized in the treatment of infectious diseases. In addition, antimicrobial drugs are in some cases associated with adverse effects on the host including excessive touchiness, insusceptible concealment and allergic reactions. Research on new antimicrobial substances should in this manner be preceded and all conceivable strategies ought to be investigated. Other than small molecules from medicinal chemistry, natural items are as yet major wellsprings of innovative therapeutic agents for various conditions, including infectious diseases [1].

The example of overcoming adversity of chemotherapy along these lines lies in the constant search for new drugs to counter the challenge postured by resistant strains of microorganisms. Ebb and flow research on natural molecules and items primarily concentrates on plants since they can be sourced all the more easily and be chosen on the basis of their ethno-medicinal utilize. Medicinal plants, which shape the backbone of traditional medicine, have in the last couple of decades been the subject of extremely serious pharmacological investigations. In this association, higher plants keep on being a rich wellspring of therapeutic agents since they create hundreds to thousands of various chemical compounds as secondary metabolites with various biological activities. The compounds created by plants are active against plant and human pathogenic microorganisms. The remarkable commitment of plants to the drug business was

conceivable, because of the large number of phytochemical and biological investigations carried out all finished the world [2].

Herbal remedies utilized as a part of the society medicine give an intriguing and still largely unexplored hotspot for the creation and improvement of potentially new drugs for chemotherapy which may help defeat the developing issue of resistance and also the toxicity of the as of now available commercial antibiotics. From an estimated 250,000 higher plants on the planet, just 5-15 % have been contemplated for a potential therapeutic value. Countless species remain yet to be investigated. Consequently it is of great enthusiasm to carry out screening of the unexplored plants with a specific end goal to validate their utilization in people medicine and to reveal the active rule by isolation and characterization of their constituents [3].

Initial screening of plants for conceivable antimicrobial activities typically starts by utilizing rough aqueous or alcohol extraction and can be trailed by various organic extraction methods. Since nearly all of the distinguished parts from plants, active against microorganism are aromatic or saturated organic compounds, they are often obtained through initial ethanol or methanol extraction. The screening of plant unrefined extracts and plant items for antimicrobial activity have demonstrated that higher plants speak to a potential wellspring of novel antibiotic models. Validation and determination of primary screening assays are pivotal to guarantee sound choice of extracts or molecules with relevant pharmacological action and commendable development [4].

2. ANTIFUNGAL SCREENING

Material & Methods

Plant selection

The primary screening of 108 plant species for antibacterial property yielded many active plant extracts. Amongst them, 20 most active plant extracts were chosen for additionally screening for antifungal property. They chose plants are recorded in Table 1.

Preparation of crude plant extract

Ten grams of dried plant material was extracted with 100 ml of methanol and kept on a rotary shaker for 24 h at the room temperature. Thereafter it was separated and centrifuged at 5000 rpm for 15 min. The supernatant was gathered and evaporated to dryness to give the unrefined dried extract. The extractive yield (%) of all the plant extracts is appeared in Table 1.

Fungal strains used

The investigated fungal strains are distinguished strains and were obtained from the National Chemical Laboratory (NCL), Pune, India. The test fungal strains incorporate 7 yeasts viz.

Candida albicans (1) ATCC2091, Candida albicans (2) ATCC18804, Candida glabrata NCIM3448, Candida tropicalis ATCC4563, Cryptococcus luteotus ATCC32044, Cryptococcus neoformans ATCC34664, Trichosporonbeigelli NCIM3404, and 4 molds viz. Aspergilluscandidus NCIM883, Aspergillusflavus NCIM538, Aspergillusniger ATCC6275 and Mucorheimalis NCIM873. The fungal strains were developed on sabouraud soup and maintained on MGYP slants (yeast) and potato dextrose agar slants (form) at 4°C.

Assay for antifungal activity

Preparation of inoculum

The test fungal strains were inoculated into sabouraud dextrose juices and incubated at 28°C on a rotary shaker. The inoculum estimate was maintained according to the 0.5 McFarland standard (1x10 cfu/ml). The activated inoculum was utilized for antifungal assay.

Preparation of test compound

The methanol extracts of chose plant species were weakened in 100 % dimethylsulphoxide (DMSO) and the stocks were prepared at the concentration of 25mg/ml, 12.5 mg/ml and 6.75 mg/ml. The antifungal activity was evaluated at three distinct concentrations viz. 500 µg/circle, 250 µg/plate and 125 µg/circle.

Antifungal susceptibility testing

The screening of methanol extracts of chose plant species for antifungal activity was dictated by agar plate dispersion strategy [5]. The liquid sabouraud dextrose agar media (Hi-Media) was 8 inoculated with 200 µl of the inoculum (1x10 cfu/ml) when the temperature of media reached 40-42°C and then filled the Petri plate (Hi-Media). Sterile circle (7 mm) (Hi-Media) was saturated with 20 µl of the extract with the concentration of 500 µg/plate, 250 µg/circle and 125 µg/circle and allowed to dry. The circle was then presented on the upper layer of the seeded agar plate. For each fungal strain, controls were maintained where unadulterated solvents were utilized instead of the extract. The plates were incubated at 28°C for 48 h. The consequence of antifungal activity was obtained by measuring the diameter of the zone of hindrance. The examination was performed under strict aseptic conditions for three times to limit blunder and the mean values are exhibited in Tables 2.4a and 2.4b.

3. RESULTS AND DISCUSSION

Antifungal screening

Fungal infections remain a significant cause of dismalness and mortality regardless of advances in medicine and the rise of new antifungal agents. Immuno-traded off patients is particularly in danger of building up these infections with Candida and Aspergillus spp. as mycoses being the

most usually distinguished. Patients who create candidemia have a greater chance of delayed hospitalization and have a mortality rate as high as 60%. Also the prevalence of Candida spp. that are resistant to triazole antifungal agents is increasing, making treatment choices a worry. Aspergillosis carries a 100% mortality rate if left untreated. In addition to this, there is a high rate of cryptococcal infections which can be because of the blast of acquired insusceptible lack disorder (AIDS) scourge around the world and the utilization of more strong immunosuppressive agents by increasing quantities of organ transplant beneficiaries.

Cryptococcal meningitis, the most widely recognized disease of cryptococcosis is usually ceaseless and consistently fatal if untreated. Some antifungal drugs, for example, polyene macrolides (amphotericin-B) and azoles (itraconazole and fluconazole) are right now utilized as a part of antifungal therapies with certain limitations because of reactions as toxicity and development of resistant strains. Although there are various treatment alternatives, no broad-range antifungal agents with an acceptable safety profile and with both intravenous and oral formulations are available at this time. These factors provoke the requirement for advancement of new antifungal agents keeping in mind the end goal to enlarge the range of activities against pathogenic fungal species and combat strains communicating resistance to the available antifungals. The methanol extracts of twenty chose medicinal plants were screened for antifungal activity against 11 fungal strains. The extractive yields of the chose plants are summarized in Table 1. The consequences of antifungal activity of screened plant species against a few strains of yeast is appeared in Table 2 and that against molds are appeared in Table 3. The antifungal activity was evaluated at three unique concentrations (500 µg/circle, 250 µg/plate and 125 µg/plate). The molds were more powerless than yeast. All the concentrations of the extracts investigated, hindered the fungal species with varying level of affectability.

Table 1 Extractive yields (%) of the plant species selected for determining antifungal activity

Plant species	Extractive yield (%)
*Ammanniabaccifera*L.	08.13
*Bauhinia variegata*L.	05.46
Caesalpiniapulcherrima(L.) Swartz.	10.27
*Casuarinaequissetifolia*Forest.	13.17
*Cyperusrotundus*L.	07.35
*Euphorbia hirta*L.	09.70
*Euphorbia tirucalli*L.	08.10
*Eucalyptus citriodora*Hook.	21.33
*Glycyrrhizaglabra*L.	11.90
Holarrhenaantidysenterica(Heyne. ex Roth.) A. DC.	15.60
*Launaeaprocumbens*Ram. & Raj.	09.43
Manilkarahexandra(Roxb) Dubard	19.98
*Mangiferaindica*L.	10.58
*Mesuaferra*L.	07.29
*Saussurealappa*Costus.	11.56

***Terminaliachebula*Retz.**	**36.92**
***Trapanatans*L.**	**02.49**
***Vitexnegundo*L.**	**13.48**
***Vitisvinifera*L.**	**07.00**
Woodfordiafruticosakurz.	**20.93**

Table 2 Screening of some Indian medicinal plants for antifungal activity against some strains of moulds

Plant species	Inhibition Zone (mm)*											
	Aspergillus candidus NCIM883			*Aspergillus flavus* NCIM538			*Aspergillus niger* ATCC6275			*Mucor heimalis* NCIM873		
	Concentration of plant extract (µg /disc)											
	500	250	125	500	250	125	500	250	125	500	250	125
***Ammanniabaccifera*L.**	-	12	-	16	11	-	-	14	11	9		
***Bauhinia variegata*L..**	11	12	10	10	20	18	18	15	13	11	12	12
***Caesalpiniapulcherrima*(L.) Swartz.**	13	11	10	10	13	15	-	12	10	10	12	-
***Casuarinaequissetifolia*Forest.**	11	-	-	-	15	16	-	13	10	12	10	13
***Cyperusrotundus*L.**	13	12	-	-	-	12	-	14	11	-	-	12
***Euphorbia hirta*L.**	9		11	-	13	14	13	11	10	9	-	-
***Euphorbia tirucalli*L.**	-	-	-	12	10	9	15	10	10	-	-	10
***Eucalyptus citriodora*Hook.**	11	-	-	17	19	21	15	15	12	9	-	-
***Glycyrrhizaglabra*L.**	16	12	11	15	19	20	13	16	14	15	14	12
***Holarrhenaantidysenterica*(Heyne. Ex Roth.) A. DC.**	11	10	10	-	10	11	15	14	16	10	10	-
***Launaeaprocumbens*Ram. & Raj.**	10	11	-	-	16	14	11	11	12	11	10	-
***Manilkarahexandra*(Roxb) Dubard**	12	-	12	20	12	17	13	15	11	-	14	10
***Mangiferaindica*L.**	12	12	-	-	-	15	12	15	13	12	-	-
***Mesuaferra*L.**	10	-	-	-	12	12	-	12	13	11	10	-
***Saussurealappa*Costus.**	10	11	-	11	10	20	13	12	11	10	10	-
***Terminaliachebula*Retz.**	10	13	10	-	15	15	14	17	16	13	-	-
***Trapanatans*L.**	9	-	-	15	12	10	12	-	12	-	-	-
***Vitexnegundo*L.**	10	11	-	-	10	11	10	10	15	10	-	-
***Vitisvinifera*L.**	10	10	-	12	10	10	-	10	16	10	-	-
Woodfordiafruticosakurz.	13	11	13	12	12	17	-	15	12	15	11	-

The overall outcomes recommend that Aspergillusflavus was the most vulnerable fungal strain and the most resistant was Candida glabrata. The aftereffects of antifungal activity of the screened plants did not demonstrate any concentration impact. Certainly indigenous plants are supplies of novel antimicrobials; they would play important parts in furnishing us with more up to date bioactive leads in future. Plants with high anti-shape activity introduced here or in similar examinations would act as bedrocks for expanding our insight to attain novel antimicrobial agents.

4. CONCLUSION

From our investigation for screening of various plant species for antibacterial and antifungal strength, the outcomes obtained affirm the therapeutic intensity of a few plants utilized as a part of traditional medicine. The outcomes obtained revealed that plant extracts in organic dissolvable ended up being more active than those in water. The plants species decided preferred antibacterial activity over antifungal activity. The most powerless bacteria were B. cereus which is a Gram-positive bacteria and the most resistant bacteria was E. coli which is a Gram-negative bacteria. A portion of the plant species namely Woodfordiafruticosakurz., Trapanatans L., Terminaliachebula Retz., Mesuaferra L., Manilkarahexandra (Roxb) Dubard, Mangiferaindica L., Eucalyptus citriodora Hook., Bauhinia variegata L., Caesalpiniapulcherrima (L.) Swartz.,Vitisvinifera L. and Euphorbia hirta L. demonstrated the best antimicrobial activity amongst all the screened plant species. Thusly, these outcomes frame a decent basis for choice of candidate plant species for encourages phytochemical and pharmacological investigation. On the basis of the outcomes evaluated for in vitro antibacterial and antifungal potencies of the chose plants, availability and literature search, Manilkarahexandra (Roxb) Dubard leaf (Sapotaceae) was chosen for advance pharmacognostic, phytochemical, toxicological and pharmacological evaluations.

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