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REVIEW ANTIFUNGAL EFFECACY OF HERBS

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Abstract

In the past few decades, a worldwide increase in the incidence of fungal infections has been observed as well as rise in the resistance of some species of fungi to different fungicidal used in medicinal practice. Besides, fungi are the one of the most neglected pathogens as demonstrated by the fact that the fluconazole and other sold treatments are still used as gold standard as antifungal therapy. The majority of used antifungal treatments have various drawbacks in terms of toxicity, efficacy as well as cost and their frequent use has also led to the emergence of resistant strains. Hence, there is a great demand for developing an antifungal belonging to a wide range of structural classes, selectively acting on new targets with least side

effects. Natural products, either as pure phytocompounds or as standardized plant extracts, provide unlimited opportunities for new drug lads because of their having normally matchless chemical diversity. Present chapter focused on the work done in the field of antifungal activities of various plant components and novel approaches which will be the future prospective for the new drug discoveries and providing better antifungal therapy.

Keywords: antifungal, phytocompounds, fungicidal, antifungal therapy,

fungal infections

INTRODUCTION

Nearly 1 in 5 adults in the United States report taking anherbal product. Written records of the use of herbal medicinedate back more than 5,000years.2 In fact, for most of history,herbal medicine was the only medicine. Even as recently as1890,59of the listings in the US Pharmacopeia were fromherbal products, and it has been estimated that as many as one third to one half of currently used drugs were originally derived from plants. Although many herbs are primarily of historical interest, thousands of herbal products are available over the counterand commonly used by patients in the United States. Therefore, an understanding of the composition, regulation, safety,and efficacy of herbs may assist clinicians in advising patientsabout the use of these products.(1)

What's in an Herb?

An herb can be any form of a plant or plant product, including leaves, stems, flowers, roots, and seeds. These plants can either be sold raw or as extracts, where the plant is macerated with water, alcohol, or other solvents to extract some of the chemicals. The resulting products contain dozens of chemicals, including fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, and others.5 Because any given herb contains multiple ingredients, some manufacturers attempt to create standardized herbal products by identifying a suspected active ingredient and altering the manufacturing process to obtain a consistent amount of this chemical.(2) In the past few decades, a worldwide increase in the incidence of fungal infections has been observed as well as rise in the resistance of some species of fungi to different fungicidal used in medicinal practice. Besides, fungi are the one of the most neglected pathogens as demonstrated by the fact that the amphotericin B and other sold treatments are still used as gold standard as antifungal therapy. The majority of used antifungal treatments have various drawbacks in terms of toxicity, efficacy as well as cost and their frequent use has also led to the emergence of resistant strains. Hence, there is a great demand for developing an antifungal belonging to a wide range of structural classes, selectively acting on new targets with least side effects. Natural products, either as pure phytocompounds or as standardized plant extracts, provide unlimited opportunities for new drug lads because of their having normally matchless chemical diversity.(3) Plant kingdom has always been a hub for many natural compounds with novel structure and this keep the investigators interested in doing research about many plants species till today. Results of new researchers showed that plants are enrich of many bioactive secondary metabolites such as saponins, alkaloids and terpenoids which characterized by antifungal

property. Depending on that, these plants can be considered as a potent future source for anti-fungal drugs [3]. When recent scenario regarding fungal diseases and antifungal drugs are taken into consideration it has been seen that the development of resistance of fungus towards the presently used antifungal drugs has increased. With the challenges like morbidity and mortality there always lies difficulty in antifungal treatment for patients receiving therapy for AIDS, diabetes, chemotherapy or organ transplant as some of the molecular processes of fungus are similar to humans, so toxicity to fungal cells could affect human cells too.(4)

In the last 30 years few drugs have made an impact in the treatment of fungal infection, one of them is amphotericin B which is among the few fungicidal drugs present antifungal therapy has but it also showed several critical side effects. In addition to this, during the period between late of 1980s and the beginning of 1990s emergence of Imidazoles and Triazoles was seen. These classes of drugs were efficient in inhibiting processes associated with fungal cells.(5) Concern has been expressed about the rising prevalence of pathogenic Properties. The different parts used include root, stem, fruit, twigs exudates and modified plant microorganisms which are Resistant to the newer (or) modern antibiotics that have been produced in the last three decades . Also, the problem posed by the high cost, adulteration And increasing toxic side effects of these synthetic drugs coupled with their inadequacy in Diseases treatment found more especially in the developing countries cannot be over emphasized Coincidentally, the last decade has also witnessed increasing intensive studies On extracts and biologically active compounds isolated from plant species used for natural On extracts and biologically active compounds isolated from plant species used for natural Therapies or herbal medicine Represent a rich source of antimicrobial agents. Plants are used medicinally in different countries And are a source of many potent and powerful drugs Medicinal plant parts is used for extract as raw drugs and they possess varied medicinal Organs. While some of these raw drugs are collected in small quantities by the local Communities and folk healers for local used, many other raw drugs are collected in larger Quantities and traded in the market as the raw material for many herbal industries . Considering the vast potentiality of plant as sources for antimicrobial drugs with reference to Antibacterial and antifungal agents, a systematic investigation was undertaken to screen the local Flora for antifungal activity from Lawsonia inermis, Mimosa pudica, Phyllanthus niruri, Tephrosia purpurea and Vinca rosea.(6)

MATERIALS AND METHODS

Materials and methods show similarity in studies of efficacy of herbs and include general steps :

1- **Plant collection and preparation of extracts**
2- **Collection of pathogens and isolates**
3- **Preparation of the media**
4- **Assessment of anti-fungal properties**
5- **Minimum inhibitory concentration assay (MIC)**

1.Plant collection and preparation of extracts : Plant extraction is a process that aims to extract certain components present in plants. It is a solid/liquid separation operation: a solid object (the plant) is placed in contact with a fluid (the solvent). The plant components of interest are then solubilised and contained within the solvent. The solution thus obtained is the desired extract. The solvent will eventually be eliminated to isolate the plant extract. If it is for the food industry, it is not necessary to separate it from the extract. If not, a second separation operation makes it possible to obtain a dry extract.

2.Collection of fungi and isolates : For isolation, fungal media with antibiotics should be used to suppress bacterial contamination. Sabouraud's dextrose agar and potato-dextrose agar are commonly used media. Cultures are routinely incubated at 25° to 30° C for up to 4 weeks.

3.Preparation of the media : There are many types cultures media for isolates fungi **including** agar , Sabouraud 2 agar , Sabouraud Chloramphenicol Actidione agar and Sabouraud Dextose agar

4.Assessment of anti-fungal properties : By the disk diffusion method .The disk diffusion method (DDM) is classified as an agar diffusion method (ADM) because the plant extract to be tested diffuses from its reservoir through the agar medium seeded with the test microorganism. Generally, the reservoir is a filter paper disk, which is placed on top of an agar surface.

5.Minimum inhibitory concentration assay (MIC) : The Minimum Inhibitory Concentration assay is widely used to measure the susceptibility of yeasts to antifungal agents. In serial two-fold dilutions, the lowest conc. of antifungal drug that is sufficient to inhibit fungal growth is the MIC. To facilitate visualization of antifungal susceptibility data, heat maps aregenerated whereby optical density values are represented quantitatively with colour.

Carica papaya

-The fresh leaves of Caricia papaya were procured.
-The samples were washed, sun dried and made into a powder .
-Leaves extracted with solvent of aqueous and 70 % ethanol.
-The solutions were filtered and left in hot air oven at 50o c till the extract got dried.
-The dried extracts were dissolved in dimethylsulfoxide, making extracts of different concentrations.

Piper betle and Ocimum sanctum Linn

- Fresh leaves of betel and Tulsi were collected.
- All the leaves were washed and then shade dried.
- The leaves were grounded into a fine powder.
- Plant extracts by solvents such as ethanol and ethyl acetate.
- It was then filtered, and the filtrate was collected.
- The ethanolic and ethyl acetate extracts were concentrated at 40°C.
- The residue was collected and stored at −20°C until further use. Three concentrations (100, 500 and 1000 µg/ml) of the extracts were prepared.

Lawsonia inermis, Mimosa pudica, Phyllanthus niruri, Tephrosia purpurea and Vinca rosea.

- The plants were collected.
- Fresh and healthy leaves were taken For each solvent extraction.
- They were washed with distilled water for three times.
- Then surface Sterilized with 0.1% mercuric chloride for 20 seconds. Again the leaves were washed thoroughly With distilled water.
- Two grams of sterilized plant leaves were kept in the 10 ml organic solvents such as n-butanol, Methanol and aqueous. Then they were ground well.
- It was Filtered through filter paper.
- The supernatant was collected by adding sterile n-butanol, methanol and aqueous stored for further antimicrobial Screening purpose.

Assessment of anti-fungal properties

Agar disc diffusion method

Antifungal activity of the sample was determined by disc diffusion method on sabouraud dextrose agar (SDA) medium. Sabouraud dextrose agar (SDA) medium was poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension.

Carica papaya leaf extract was used and cultured again. The

antifungal activity was determined by measuring the diameter of zone of inhibition (mm) around the disk, which was measured by vernier caliper. One mg of plant extract powder was taken and mixed with 1 ml of DMSO obtaining the concentration of 1 mg/ml.

MIC determination:

This assay determines the capability to inhibit the growth of fungi. One ml of sterile potato dextrose agar broth was distributed to 8 tubes and was submitted to autoclave under constant pressure at the temperature of 121 C After the broth reaches room temperature, 1 ml of diluted sample was added in tube1. Then 1 ml was transferred from tube 1 to tube 2. This transfer was repeated successively until it reaches tube 8. 100 µl of Candida albicans cultures were added to all the tubes from 1 to 8. Incubation was done at 37C for 24 hrs. After incubation, the turbidity was observed. MIC, the conc. of higher dilution tubes in which the absence of fungal growth occurred, were noted.

Piper betle and Ocimum sanctum Linn

The fungal strains were maintained on potato dextrose agar medium. A loopful of culture from the slant was inoculated into the medium and incubated at 28°C for 48–72 h, and 0.1 ml of this culture was evenly spread on the plates containing respective media. Filter paper of about 6-mm diameter were impregnated on the surface of the media. Different concentrations of both extracts were prepared and applied on the discs and incubated for 48 h at 28°C.

The results were recorded by measuring the inhibition zone around the discs [Figures 1-3].

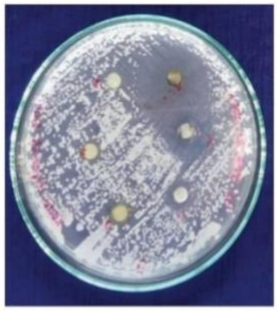

Figure 1: Ethanolic and ethyl acetate extracts of young betle leaf showing zone of inhibition

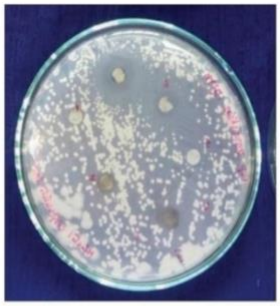

Figure 2: Ethanolic and ethyl acetate extracts of mature betle leaf showing zone of inhibition

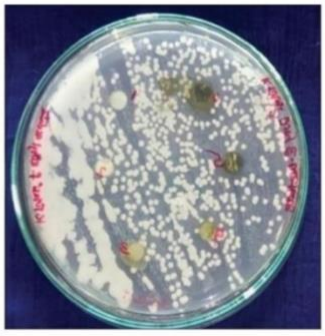

Figure 3: Ethanolic and ethyl acetate extract of tulsi leaf showing zone of inhibition

Determination of MIC : The lower the concentration required, the higher is the efficacy. An extract concentration of 0.1–2 mg/ml was evaluated. Specifically, 0.1 ml of standardized inoculum (1–2 × 107 colony-forming unit/ml) was added to each test tube. The tubes were incubated aerobically at 28°C for 48–72 h. Two controls were maintained for each test sample. The lowest concentration (highest dilution) of the extract that produced no visible signs of microbial growth (no turbidity).

Lawsonia inermis, Mimosa pudica, Phyllanthus niruri, Tephrosia purpurea and Vinca rosea.

Antifungal activity was screened by agar well diffusion method. The n- Butanol, methanol and aqueous extracts of five different medicinal plants were tested against Plant pathogen *Pythium debaryanum*. The PDA medium was poured in to the sterile petriplates and allowed to solidify. 200µl of Each extracts were transferred into the separate wells. The plates were incubated at 27°C for 48 – 72 hrs. After the incubation the plates were observed for formation of clear incubation zone Around the well indicated the presence of antifungal activity.

Results and Discussion

The leaf extract of Carica papaya against C.albica and its synergy

with fluconazole As evident from (Figure 1 to 4) and summarised in (Table 1).The antifungal activity was not demonstrated with aqueous extract of papaya leaf extract with increasing concentrations. In ethanolic extract of plant, demonstrated the antifungal activity against Candida albicans, of which

highest concentration showed significant activity with zone diameter of

11.97±0.15 mm (Table 1).

Table 1: Zone of inhibitons of test and standard drug.

S. no.	Test	Zone of inhibition (mm) (Mean±SD)			
		1000 (µg/ml)	750 (µg/ml)	500 (µg/ml)	Fluconazole (1mg/ml)
1	Papaya leaf ethanol extract	11.97±0.15	9.7±0.7	8.73±0.59	12.93±0.66
2	Papaya leaf ethanol extract + Fluconazole	13.6± 0.45	9.3±0.56	8.2±0.60	12.83±0.95
3	Papaya leaf aqueous extract	-	-	-	14±0.3

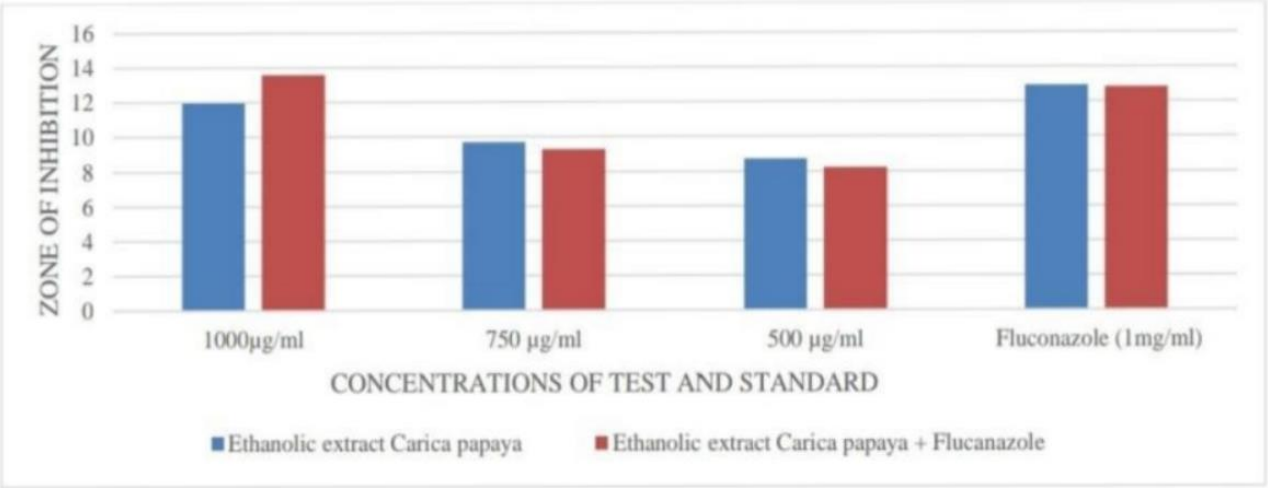

Figure 1: Comparing the zone of inhibition of ethanoolic *Carica papaya* leaf extract and ethanolic *Carica papaya* leaf with fluconazole.

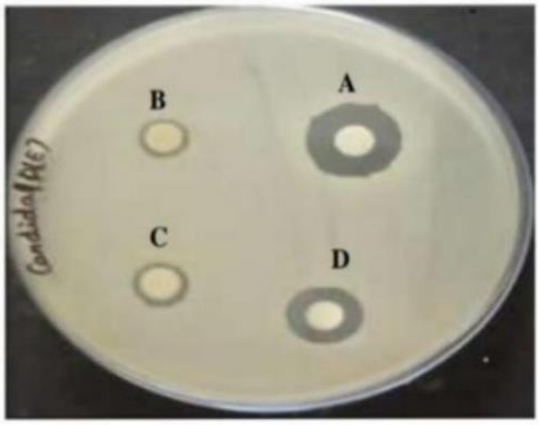

Figure 2: The zone of inhibition of ethanolic extract of *Carica papaya* leaf in diffrent concentrations. A- Fluconazole, B-500 µg/ml of ethanolic plant extract, C-750 µg/ml of ethanolic plant extract, D- 1000 µg/ml of ethanolic plant extract.

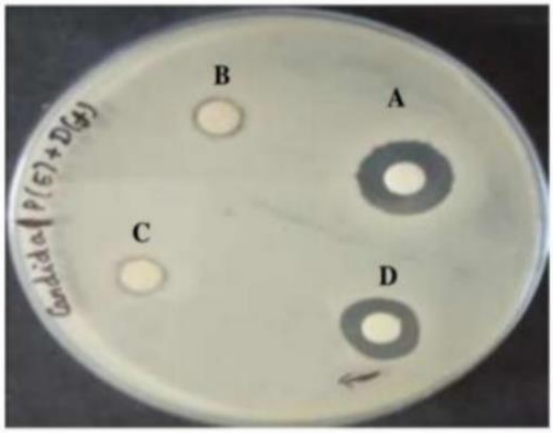

Figure 3: The zone of inhibition of ethanolic extract of *Carica papaya* leaf + Fluconazole in diffrent concentrations. A- Fluconazole, B-500µg/ml of ethanolic plant extract+ Fluconazole, C-750µg/ml of ethanolic plant extract+ Fluconazole, D- 1000µg/ml of ethanolic plant extract+ Fluconazole.

The synergistic activity with Fluconazole and plant extract showed highly significant antifungal activity with zone of inhibition of 13.6±0.45mm when compared with the standard drug (fluconazole) (Figure 3).

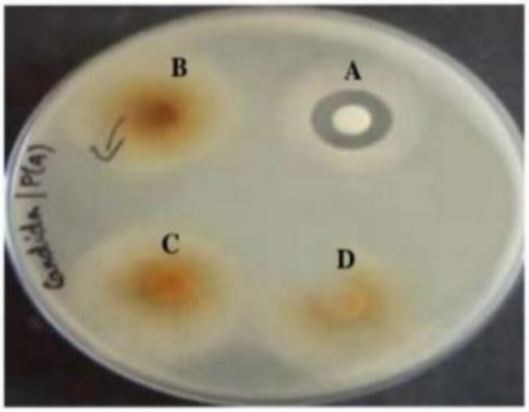

Figure 4: The zone of inhibition of aqeous extract of Carica papaya leaf. A- Fluconazole, B-500 µg/ml of aqeous plant extract, C-750 µg/ml of aqeous plant extract, D- 1000 µg/ml of aqeous plant extract.

Table 2: Minimum inhibitory concentrations of test and standard.

S. no.	Sample	Minimum inhibitory concentration (µg/ml)
1.	Fluconazole (drug)	500
2.	Papaya leaf ethanol extract	350
3	Papaya leaf ethanol extract+Fluconazole	125
4	Papaya leaf aqueous extract	-

Minimum inhibitory concentration

In this test, the plant extract showed moderate antifungal activity with MIC of 250µg/ml when compared to the control. This test also demonstrated the Synergistic activity of the antifungal property of the extract with MIC of 125µg/ml (Table 2). The aqueous extract did not show any activity. Recent years emphasize the need for antifungal agent in the current situation to Overcome the eminent crisis due to increased incidence of resistance and Antifungal treatment failures. The major causative factor for the development Of this crisis owes largely to increase in the immune compromised population. And need for prolonged therapy in situations such as HIV patients, organ Transplantation and cancer chemotherapy .In this current study, the antifungal activity of the papaya leaf ethanolic extract Was clearly demonstrated in all concentrations, when compared to standard. This result was seen in both disc diffusion assay and minimum inhibitory Concentration which was in agreement to a study in which revealed that the Papaya leaf extract exhibited antifungal property in well diffusion method. Our study showed that the concentration of ethanolic extract effectively Suppresses the mycelia growth of Candida albicans and this effect was found to Increase with concentration of ethanolic extract. Although, synergistic activity Of the Carica papaya leaf extract with the standard drug Fluconazole has not Been studied previously. The current study reveals that the papaya leaf extract Markedly reduces the MIC, which indicates that the minimum amount of the Test components is needed to inhibit the Candida growth, when used along with Fluconazole. Also, the zone diameter was increased when used in combination, Stipulating Suppresses the mycelia growth of Candida albicans and this effect was found to Increase with concentration of ethanolic extract. Although, synergistic activity Of the Carica papaya leaf extract with the standard drug Fluconazole has not Been studied previously. The phytochemical constituent of the medicinal plants plays a Major role in its therapeutic potential. In a study the phytochemical components Of Carica papaya have been studied and they showed many active principles Such as alkaloids, carbohydrates, saponins, glycosides, proteins and aminoacids,Phytosterol, flavinoids, terpinoids and tannins in various extracts. This study also Showed that the ethanolic extract contained all the active principles found in the Plant extract but the aqueous plant extract showed only alkaloids. In our study, this may attribute to no activity of aqueous plant extract and presence significant antifungal activity in ethanolic plant extract. Also, it may be safe to to the presence of more amount of active principle in

higher concentration of the ethanolic extract of Carica papaya. However, further research is needed to unveil the mechanism of action and the specific active component of the extract contributing to the antifungal activity .From this study, we found that the Carica papaya leaf extract has an antifungal activity and has synergistic effect when used with fluconazole. Further, in-vivo studies with other fungi will assess the potential use of these compounds for Extended therapeutic applications.From this study, we can safely conclude that the Carica papaya leaf extract has A significant antifungal property and exhibit synergistic effect when used with Fluconazole. Therefore, this can be considered as a potential agent against Human pathogenic fungi in future after meticulous research. This preliminary Study was an attempt with positive results and a bridge for future research to Study was an attempt with positive results and a bridge for future research to Develop a potential agent to overcome the emerging public health crisisExtract of piper betle and ocimum sanctum against Candida albicans and its synergy with fluconazole The anticandidal activity of ethanolic and ethyl acetate extracts of betel and Tulsi leaves along with standard drug fluconazole was analyzed in the present Study, and the results are tabulated [Tables 1-3]. The zone of inhibition Increased in a dose-dependent manner. Among the three concentrations (100, 500 and 1000 µg/ml) used, the maximum inhibitory zone was observed at 1000 µg/ml in the ethyl acetate extract of mature leaves(26 mm), followed by tulsi and fluconazole (13 mm). This was twice as better in betel leaf than the other two.The MIC of ethanolic and ethyl acetate extracts of betel and tulsi leaves along with standard drug fluconazole was tested, and the results are shown in Tables 3 and 4. Fluconazole showed the least MIC value (62.5 µg/ml) followed By ethyl acetate extract of betel (125 µg/ml) and tulsi (2000 µg/ml) leaves.

Table 1: Anticandidial activity of betel leaf extract

Concentration (µg/ml)	Zone of inhibition (mm)			
	Ethanolic extract		Ethyl acetate extract	
	Young	Mature	Young	Mature
100	-	5	10	-
500	8	12	22	17
1000	15	22	22	26

Table 2: Anticandidial activity of tulsi leaf extract

Concentration (µg/ml)	Zone of inhibition (mm)	
	Ethanolic extract	Ethyl acetate extract
100	-	-
500	7	-
1000	13	13

Table 3: Minimum inhibitory concentration of betel and tulsi leaf extracts

Extract	MIC (µg/ml)		
	Betel leaf		Tulsi leaf
	Young	Mature	
Ethanol	500	750	2000
Ethyl acetate	250	125	2000

MIC: Minimum inhibitory concentration

Table 4: Anticandidial activity of standard drug

Drug	Zone of inhibition (mm)	MIC (µg/ml)
Fluconazole	13	62.5

MIC: Minimum inhibitory concentration

According to the results obtained in the current study, the ethyl acetate Extract of mature leaves of P. betle showed the maximum inhibitory zone Against C. albicans (26 mm). This was twice larger than the inhibitory zone Obtained from tulsi and standard drug fluconazole (13 mm). A study by Nanayakkara et al. revealed that extracts from young betel leaf Showed higher anticandidal activity than that of mature leaves. This was Explained by the release of more secondary metabolites from young leaves from young leaves because of the fragility of their physical defenses such as lack of a thick waxy Cuticle and low cell wall rigidity. Our study showed contrary results wherein Mature leaves had better anticandidal activity than young leaves, which can Probably be explained by the use of ethyl acetate, a solvent not used in the Study by Nanayakkara et al., hence an increased release of secondary Metabolites in mature leaves. However, this requires more studies to confirm The effect of ethyl acetate solvent in the increased release of secondary Metabolites from betel leaf.In the current study, the ethanolic and ethyl Acetate extracts of tulsi leaves showed half the amount of inhibitory zone Against C. albicans (13 mm) when compared with betel leaf and the equal Amount with standard drug fluconazole. To the best of our knowledge, this was The first study to examine the anticandidal activity of betel leaf and tulsi with An approved drug. The low candidal activity of the tulsi leaf may be improved Via the use of different solvents and different extraction procedures, Considering the polarity of the active compounds. In addition, the Purification of the active compound may lead to higher anticandidal activity. Hydroxychavicol present in betel leaf extract and methyl chavicol and linalool In tulsi leaf extract are responsible for their anticandidal activity. They alter the Cell membrane structure, resulting in the disruption of the permeability barrier Of microbial membrane structure .Phytochemical analysis in our laboratory Also revealed the presence of these secondary metabolites. Further, the efficacy of these leaf extracts was tested by obtaining their MIC Values. It showed that the MIC value of fluconazole (62.5 µg/ml) was twice Better than those of ethyl acetate extract of mature betel leaves(125 µg/ml). In Similar MIC values were observed against C. albicans as reflected in our study.

The results of this study demonstrate that the ethyl acetate extract of P. betle Is more effective as an anticandidal agent against C. albicans when compared With tulsi and fluconazole. This would help to replace drugs to which Candida Have evolved resistance by promoting these traditional medicines. Further

Studies on tulsi by using different solvents would increase the efficacy of this Plant product compared to standard drug. Effect of antifungal activity of Some medicinal plants against Pythium debaryanum. Antifungal activity of five medicinal plants extract was Assayed by agar well diffusion method. The result revealed that the Extract of five medicinal plants showed significant reduction in Growth of P. debaryanumAmong all the five plants extract the n-butanol and methanol extract of Lawsonia inermis Exhibited maximum antifungal activity (15 and 20 mm) followed by Phyllanthus niruri (15 and 20 mm), Tephrosia purpurea (10 and 15 mm) P. debaryanum. The methanolic extract of Mimosa pudica (20 mm), Vinca rosea (10 mm) exhibited least activityagainst P. debaryanum. The results of antifungal effect of aqueous Extract of all tested five plants showed no activity Against P.Debaryanum. (Table1) The methanol leaf extracts of various medicinal plants showed significant Antibacterial and Antifungal activity against Aspergillus flavus, Dreschlera turcica and Fusarium verticillioides Have been reported (2008) Methanolic extracts of Tephrosia purpurea showed excellent antibacterial And antiviral activity extracts from fruits of Schisandra chinensis Separated into n-butanol and Diethyl ether showed antagonistic effects on Alternaria alata. Methanolic Extracts of root and shoots of the herb Heracleum candicans wall (Apiaceae), showed antifungal extracts of Root and shoots of the herb Heracleum candicans wall (Apiaceae), Showed antifungal Effect against Pythium and Aspergillus species only. Aqueous and chloroform shoot extracts and Aqueous root extract did notshow Show any antifungal effect against the Alternaria, Aspergillus, Fusarium, Penicillium, Phytophthora and Pythium have also been reported (2006)

Table 1 Effect of Antifungal activity of some medicinal plants against *Pythium debaryanum* (Hesse)

S. No.	Medicinal Plants	Zone of Inhibition (mm)		
		n-butanol	Methanol	Aqueous
1.	*Lawsonia inermis*	15	25	-
2.	*Mimosa pudica*	-	20	-
3.	*Phyllanthus niruri*	15	20	-
4.	*Tephrosia purpurea*	10	15	-
5.	*Vinca rosea*	-	10	-

Q/In the current studies, why are researchers moving towards herbs that have the property of antifungal activity?

Ans/ Recent years emphasize the need for antifungal agent in the current situation to overcome the eminent crisis due to increased incidence of resistance and antifungal treatment failures. The major causative factor for the development of this crisis owes largely to increase in the immune compromised population and need for prolonged therapy in situations such as HIV patients, organ transplantation and cancer chemotherapy.

Q// What mean the MIC and how can determine?

Ans/ Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that appears to inhibit the growth of the microbes. The lower the concentration required, the higher is the efficacy. MIC was determined by a microbroth dilution method according to the Clinical and Laboratory Standards Institute guidelines.

Q//A study by Nanayakkara et al. revealed that extracts from young betel leaf showed higher anticandidal activity than that of mature leaves

Whereas Our study showed contrary results wherein

mature leaves had better anticandidal activity than young leaves? Explain it.

Ans/ Because study by Nanayakkara et al. revealed that extracts from young betel leaf showed higher anticandidal activity than that of mature leaves this is explained by the release of more secondary metabolites from young leavesbecause of the fragility of their physical defenses such as lack of a thick waxy cuticle and low cell wall rigidity while in our study showed contrary results wherein mature leaves had better anticandidal activity than young leaves this probably be explained by the use of ethyl acetate, a solvent not used in the study by Nanayakkara et al. hence an increased release of secondary

Q//How can improved the anticandidal activity of tulsi leaf?

Ans/ The low anticandidal activity can be improved via the use of different solvents and different extraction procedures, considering the polarity of the active compounds. In addition, the purification of the active compound may lead to higher anticandidal activity.

Q//What is the responsible for anticandidal activity of betal leaf extract and tulsi leaf extract and by which mechanism it act?

Ans/ Hydroxychavicol present in betel leaf extract and methyl chavicol and linalool in tulsi leaf extract are responsible for their anticandidal activity. They alter the cell membrane structure, resulting in the disruption of the permeability barrier of microbial membrane structure.

Q/ why betal leaf and tulsi leaf extract have Higher MIC when compared with standard drug (flucanazole) in our study?

Ans/ This is can be attributed to the use of crude extracts of the leaves when compared with the purified form of the standard drug.

Q/ what mean by agar diffusion method?

Ans: Agar diffusion. Also known as agar contact method, it is the least-employed one of the techniques. It involves the transfer by diffusion of the antimicrobial agent from the chromatogram (PC or TLC) to an agar plate previously inoculated with the microorganism tested

Q/ We used five medicinal plants, which any extract of them exhibit maximum antifungal activity?

Ans:The extract the n-butanol and methanol extract of Lawsonia inermis Exhibited maximum antifungal activity (15 and 20 mm) followed by Phyllanthus niruri (15 and 20 mm), Tephrosia purpurea (10 and 15 mm) P. debaryanum. The methanolic extract of Mimosa pudica (20 mm), Vinca rosea (10 mm) exhibited least activity against P. debaryanum .

Q/ what the type of agar used in article 3 and from which consist?

Ans : potato dextrose agar and consist of potato, dextrose, agar and D.W .

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