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IN VITRO ANTIMICROBIAL ACTIVITY OF SOME PLANTS AGAINST HUMAN PATHOGENIC FUNGI

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ABSTRACT

In traditional medicine of India, around 40% of herbal plants are used to treat diseases caused by pathogenic fungi. Medicinal plants are common in India; many plant species potentially useful for new pharmaceuticals are less studied. However, the antimicrobial properties of several medicinally important plants from various countries are still unknown. This paper aims to provide report on the antimicrobial activity of *Datura mate and Butea monosperma* medicinal plants used again Human pathogenic microbes as traditional medicine. A total of 8 fungal pathogens were isolated from various diseased samples of human body. *Aspergillus niger* (FCHP#05) was the most fungal sp. Isolated accounting for 43% followed by *Aspergillus flavus* (FCHP#06) 32%, *Candida* (FCHP#04) 26%, *Tricophyton* sp. (FCHP#02) 22%, *Aspergillus fumigates* (FCHP#07) 16%, *Microsporum* sp. (FCHP#01) 12%, *Cladosporium* sp. (FCHP#08) 11% and *Epidermophyton* sp (FCHP#03) 08%. Alcoholic leaves extract of *Cassia fistula, Datura mate and Butea monosperma* spp. was subjected to antifungal activity by agar well method and disk diffusion method. Maximum percentage inhibition was observed with 7 Days of incubation viz. *Epidermophyton* sp., *Tricophyton* sp., and minimum percentage inhibition was observed. *Candida* followed by *Aspergillus niger* by agar well and disk diffusion method.

KEYWORDS: Antifungal activity, Plants, Human pathogenic fungi.

INTRODUCTION

The main function of antimicrobial agents is to lessen the burden of infectious diseases worldwide (Bhatia *et al*., 2010). However, because there are fewer, or occasionally no, effective antimicrobial treatments available for the illness caused by pathogenic microbes, the introduction and spread of multidrug resistant (MDR) strains in pathogenic microbes have become a substantial public health threat [Bradley *et al*, 2009, Giamarellou 2010]. Numerous medicinal plants have been identified as important sources of naturally occurring antimicrobial chemicals as a potential substitute that may be successful in the treatment of these troublesome bacterial illnesses. [Holguin et al 2005] Due to their antibacterial properties, which are brought on by phytochemicals produced during the plant's secondary metabolism, many plants have been employed [Romero et al 2005]. Plants contain a wide range of secondary metabolites, including flavonoids, phenolic compounds, alkaloids, and tannins, which have been shown to have antibacterial activities in vitro [Duraipandiyan *et al* 2006, Seukep *et al* 2013].

There are many fungi responsible for skin diseases that can affect Ringworm of the skin, Ringworm of the Scalp, Nail Infection and hair. (Auroba *et al*. 2012) Mycosis, infections are probably the most common cause of skin disease in developing countries of tropical regions. Dermatophytosis is the most frequent superficial fungal infection occurring in India. The remedies of derived from natural resources are widely used to treat of dermatology disease problems, as age-old tradition (McChesney *et al*. 2007). It is estimated that various plant species were screened for medicinal properties and used by 80% of the world's population to treat human fungal diseases (Saslis-Lagoudakis *et al*. 2014; Chen *et al*. 2016). The use of medicinal herbal drugs in the treatment of skin diseases including mycotic infections is an age-old practice in many parts of the world. This use has been supported by the isolation of active antifungal compounds from plant extracts (Costa *et al*. 2002). Since the cost of synthetic, medicinal drugs is high, the developing countries are still using herbal plants or their derivates to treat common diseases. The knowledge of social-demographic background of individuals infected with skin infections has not been the focus of most sub-Sahara disease control programs impacting negatively to the well being of the individuals at risk (Chepchirchir *et al*. 2009). The purpose of this study was to determine the fungal species causing skin infections among patients and investigate the antimicrobial activity of like *Cassia fistula, Datura mate and Butea monosperma*.

MATERIAL AND METHODS

Method of sample collection, isolation and maintenance of culture

Fungal infected skin scraping was collected from various patients who have attended dermatology clinic at Jabalpur, (M.P.) Infected Skin were collected through Sterile scalpel and skin scraping were collected in prepared sabouraud agar media. In a few test tubes, slants of Sabouraud agar containing polypeptone agar and glucose were obtain used for fungal culture. The cultures were incubated at 28-30°C in BOD incubator for 5-7 days. When the fungal colonies have grown on the agar surface the surface of the medium is observed first through the glass of the culture tube. The fungal species isolated earlier were purified by streak-plate and sub culturing techniques (Agarwal & Hasija, 1986) and brought to pure culture by single spore culture, prepared with the help of dummy cutter objective. The stock cultures of the microorganisms were maintained on the PDA slants.

Microscopic studies of pathogens

Identification of fungi was done after studying the morphological and cultural characteristics with the help of monographs, manuals and papers of various workers. Slide culture technique was adopted for identification and slides were prepared with lacto phenol and Cotton blue. (Subramaniam 1971, Barnet and Hunter 1972, Ellis, 1971 and Sutton 1980).

Determination of Frequency

The frequencies of different fungi were determined by using following formula.

$$\text{Percentage (\%) Frequency of Individual Fungus} = \frac{\text{Total no. of colonies of Individual Fungus in a plate}}{\text{Total no. of different fungi in a plate}} \text{ x } 100$$

$$\text{Percentage(\%)Frequency} = \frac{T1}{T2} \text{x} 100$$

Collection of Plant Material and their extraction

Collection of healthy leaves sample from plant like *Cassia fistula* (S1), *Datura mate* (S2) *and Butea monosperma* (S3) from different location of Jabalpur**,** M.P. About 500g leaves from trees of the *Cassia fistula, Datura mate and Butea monosperma* were collected and air-dried then 10 gm of *Cassia fistula, Datura mate and Butea monosperma* powder was subjected to hydro-distillation for 6-8 hours using a Clevenger-type apparatus (Shibamoto, 1989). Cycles should be done 6-10 times and extract was recovered by filtration and Extracts were concentrating into 30% by rotavapour for further analysis. (Avnish *et al.*, 2020).

***In vitro* assay**

Evaluated of the antifungal activity of medicinal plants extracts against dermatophytes. This will be done by agar well and Disk-diffusion method.

Agar well method

Potato dextrose agar media growth media were used for fungi. 50 µl of the different fungal cultures were spread into the plates using a sterile spreader. The plates were punch with 6 mm diameter wells and filled with 25 µl of the plant extract and amphotericin (100 µg/ml) was used as controls. The tests were carried out in triplicates. The fungal plates were incubated at 28°C. The diameter of the zone of inhibition was measured in millimeters at 96 hrs.

Disk-diffusion method

Whatman filter papers were used to prepare the disk. 25 µl of plant extracts were poured on the disk carefully and left overnight for drying. Once the agar was solidified, 50 µl of the different fungal cultures were spread onto the plates using a sterile spreader. The disk was place on the plates. Simultaneously, amphotericin (100 µg/ml) was used as positive controls. The tests were carried out in triplicates. The fungal plates were incubated at 28°C. The diameter of the zone of inhibition was measured in millimeters at 96 hrs. (Khedoudja, *et al 2020*).

The percentage of mycelial inhibition was calculated/ computated by mean value of colony diameter by the following formula:

$$\text{Percentage of mycelial inhibition} = \frac{dc - dt}{dc} \times 100$$

dc - average diameter of fungal colony in control sets.

dt - average diameter of fungal colony in treated sets.

RESULTS

During a survey of Jabalpur, it was observed that fungal nail infection, Ringworm of the Feet, Ringworm of the Scalp, Ringworm Tinea Corporis, Ringworm of the Hand, Ringworm of the Skin etc disease were associated with human body. A total of 8 fungal pathogens were isolated from various diseased samples of human body. *Aspergillus niger* (FCHP#05) was the most fungal sp. Isolated accounting for 43% followed by *Aspergillus flavus* (FCHP#06) 32%, *Candida* (FCHP#04) 26%, *Tricophyton* sp. (FCHP#02) 22%, *Aspergillus fumigates*

(FCHP#07) 16%, *Microsporum* sp. (FCHP#01) 12%, *Cladosporium* sp. (FCHP#08) 11% and *Epidermophyton* sp (FCHP#03) 08%.

Table No. 1: Human Pathogenic Fungi isolated from Human Body.

S. No.	Name of Fungi	Isolate code no.	Source	Frequency in %
1	*Microsporum* sp.	FCHP#01	Nail Infection	12%
2	*Tricophyton* sp.	FCHP#02	Ringworm of the Scalp	22%
3	*Epidermophyton* sp.	FCHP#03	Ringworm of the Skin	08%
4	*Candida*	FCHP#04	Ringworm of the Hand	26%
5	*Aspergillus niger*	FCHP#05	Nail Infection	43%
6	*Aspergillus flavus*	FCHP#06	Ringworm of the Hand	32%
7	*Aspergillus fumigatus*	FCHP#07	Nail Infection	16%
8	*Cladosporium* sp.	FCHP#08	Ringworm of the Scalp	11%

FCHP- Fungus Culture Human Pathogenic.

Antifungal activity of *Cassia fistula, Datura mate and Butea monosperma* leaves extract by Agar well method

As shown in alcoholic extract of *Cassia fistula, Datura mate and Butea monosperma* spp. leaves was subjected to antifungal activity with different fungal strains at different incubation periods. Maximum percentage inhibition was observed with 7 Days of incubation viz. *Epidermophyton* sp., *Tricophyton* sp., *Aspergillus flavus, Cladosporium* sp, *Cladosporium* sp, *Microsporum* sp. and minimum percentage inhibition was observed with 7 days incubation period viz. *Candida* followed by *Aspergillus niger*.

Table 2: Antifungal activity of Plant leaves extract by Agar well method.

S. No.	Test Organisms	Zone of inhibition		
		***Cassia fistula* (S1)**	***Datura mate* (S2)**	***Butea monosperma* (S3)**
		Alcoholic	**Alcoholic**	**Alcoholic**
1	*Microsporum*	57.5±0.30	51.3±0.18	48.6±0.22
2	*Tricophyton*	57.9±0.26	56.5±0.37	52±0.11
3	*Epidermophyton*	64.1±0.31	62.2±0.09	61.3±0.28
4	*Candida*	47.3±0.42	47.8±0.43	37.2±0.37
5	*Aspergillus niger*	51.3±0.22	52.9±0.46	40.2±0.19
6	*Aspergillus flavus*	57.1±0.02	55.3±0.05	52.1±0.12
7	*Aspergillus fumigatus*	55.1±0.02	52.1±0.04	52.1±0.02
8	*Cladosporium* sp.	56.4±0.12	53.5±0.05	53.7±0.11

(S1- Plant sample -1, S2- Plant sample -2 and S3- Plant sample -3)

Data are mean of three replicate.

Antifungal activity of *Cassia fistula, Datura mate and Butea monosperma* leaves extract by Disk diffusion method

As shown in alcoholic extract of *Cassia fistula, Datura mate and Butea monosperma* spp. leaves was subjected to antifungal activity with different fungal strains at different incubation periods. Maximum percentage inhibition was observed with 7 Days of incubation viz. *Epidermophyton* sp., *Tricophyton* sp., *Aspergillus fumigates, Cladosporium* sp., *Microsporumsp., Aspergillus flavus* and minimum percentage inhibition was observed with 7days incubation period viz. *Candida* followed by *Aspergillus niger*.

Table 3: Antifungal activity of Plant leaves extract by Disk diffusion method.

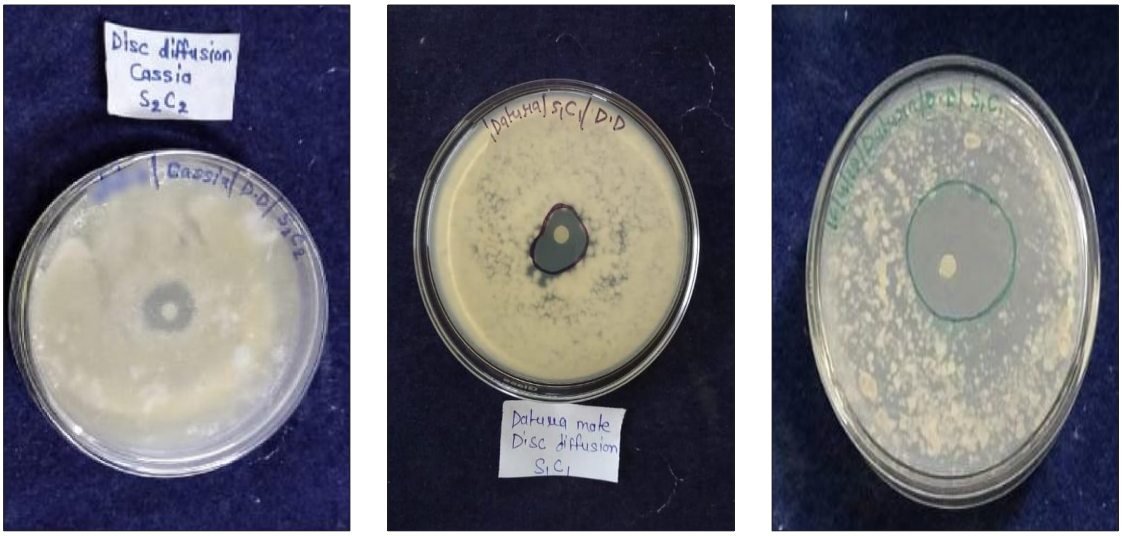
S. No.	Test Organisms	Zone of inhibition		
		***Cassia fistula* (S1)**	***Datura mate* (S2)**	***Butea monosperma* (S3)**
		Alcoholic	**Alcoholic**	**Alcoholic**
1	*Microsporum*	53.9±0.24	54.7±0.14	49.9±0.22
2	*Tricophyton*	56±0.14	54.2±0.31	56.8±0.21
3	*Epidermophyton*	65.2±0.31	61.2±0.01	60.4±0.23
4	*Candida*	49.5±0.51	42.1±0.37	41.2±0.42
5	*Aspergillus niger*	51.5±0.27	53.5±0.24	45.1±0.24
6	*Aspergillus flavus*	52.1±0.12	54.5±0.01	50.1±0.14
7	*Aspergillus fumigatus*	56.9±0.02	55.5±0.07	52.7±0.01
8	*Cladosporium* sp.	55.7±0.01	54.5±0.15	52.9±0.11

(S1- Plant sample -1, S2- Plant sample -2 and S3- Plant sample -3)

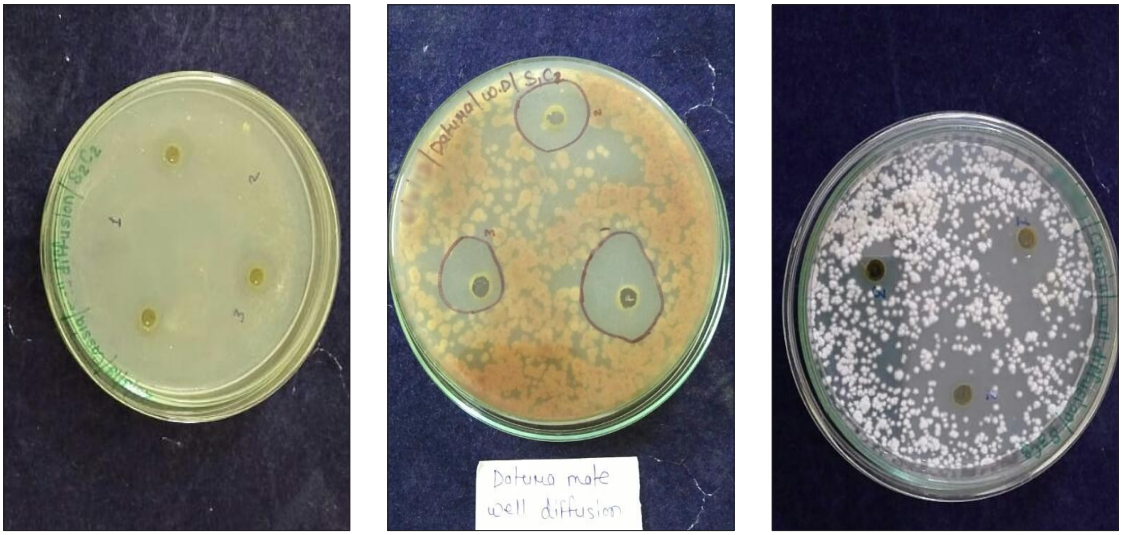
Data are mean of three replicate.

CONCLUSION

A total of 8 fungal pathogens were isolated from various diseased samples of human body. alcoholic extract of *Cassia fistula, Datura mate* and *Butea monosperma* spp. was subjected to antifungal activity by agar well and disk diffusion method. Maximum percentage inhibition was observed with 7 Days of incubation viz. *Epidermophyton* sp., *Tricophyton* sp., *Aspergillus fumigates, Cladosporium* sp., *Microsporumsp., Aspergillus flavus* and minimum percentage inhibition was observed viz. *Candida* followed by *Aspergillus niger*. Various contain biologically active compounds of plants, which could help discover novel drugs. The present study described the status of the plants from *Cassia fistula, Datura mate and Butea monosperma* sp. and provided antimicrobial properties and the justification for continuing search for novel drugs. The utilization of plant compound has excellent potential to discover antimicrobial properties again Human pathogenic microbes.



Anti fungal activity by Well Diffusion Method.



Anti fungal activity by agar well method.

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