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Antifungal activity of the *Eucalyptus australe* important medicinal plant.

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ABSTRACT: *The present investigations are being carried out to evaluate the antifungal medicinal properties of Eucalyptus australe. The effects of different concentrations of alcoholic extract of Eucalyptus australe(root, shoot and seed) on the radial growth of plant against the pathogenic fungi viz. Aspergillus niger, Aspergillus flavus, Candida albicans and Microsporum fulvum. That with the increase in concentrations the rate of growth inhibition also increases. Observation further shows that like root extract growth is also inhibited in the presence of shoot and seed alcoholic extract under culture medium. Further shows that the growth of these fungi inhibits more in presence of higher concentrations as compared to lower concentrations of extract.*

Keywords—*Eucalyptus australe, antifungal, alcoholic extract.*

I. INTRODUCTION

The medicinal importance of plant products is well known to man from the ancient times. Many plants possess substances of fungi static or fungicidal nature. There are 50,000 valid species of Fungi but only 50 to 100 are generally recognized as known to cause pathogenic diseases to human beings. Dermatophytes are known to cause superficial skin infections like ring worm, Tinea capitis Tiner corporis, etc in man, animal and birds. Considering the rich diversity of Indian medicinal plants it is expected that screening and scientific evaluation of certain medicinal plants extracts of Saharanpur and Shiwalik Himalayan belt for their antifungal substances may be worked out in order to benefit human beings.

Leptadenia reticulata (Retz.) Wight. & Arn. of (Asclepidaceae) is an important twining medicinal shrub possessing abundant alkaloids (Anonymous 1962). Eucalyptus is among the world's important hardwoods and principal source of timber in Australia. It is used for bridges, railway sleepers plywood industries etc in India, however, it is not used as timber, but mainly as fuel. The essential oils derived by steam distillation of leaves and branches are of commercial importance, it may be grouped under (a) pharmaceutical or medicinal oils, (b) industrial oils, and (c) perfumery oil. The oil is antiseptic and is used in infections of the upper respiratory tract and certain skin diseases. Mixed with equal amount of olive oil it is used for rheumatism and also in ointments for burns. The dried leaves used in the form of tincture and is also used in asthma and chronic bronchitis. The root is used as a purgative.

II. MATERIAL AND METHODS

Plants were collected from district Saharanpur & Shiwalik belt of Uttar Pradesh as well as from Garhwal hills of Uttranchal, India. Identification of plants was done through herbarium available in the deptt of Botany, M.S. College, Saharanpur & Forest Research Institute, Dehradun. List of plant prepared. The plant material were collected from wild areas of Saharanpur district and also of Garhwal Himalaya. The method was followed based on work of Natarajan et al. (2001) with some modifications.

For the preparation of *Eucalyptus australe*plant extracts 5 gm of fresh plant part was washed 2-3 times with distilled water & than surface sterilized with 90% alcohol. Subsequently the plant material was grounded in 50 ml of distilled water & alcohol separately for aqueous and alcoholic extracts, respectively. The alcoholic extracts were kept for 24 hrs. at room temperature to evaporate the alcohol. In the remaining residue, 50 ml of distilled water added. The macerates were squeezed through double layered Muslin cloth & filtered through filter paper. After filtration, the aliquot was centrifuged at 5000 rpm for 30 minute. The supernatants were filtered through whatmann no. 1 filter paper & then sterilized by passing through 0.2 micron disposable filters. The various concentrations of extract made & thus obtained were used in studies. initially treated with 0.1% $HgCl_2$ solution for sterilization and subsequently washed thoroughly with sterile distilled water and grounded in

motor & pistil with 50% methanol. The homogenized liquid was filtered and centrifuged at 3000 to 5000 rpm. The supernatant was used as test extract & make up into 20 ml using 50% methanol. Further, the extract was diluted into different concentrations, i.e. 10%, 25%, 50%, 75%, 20 ml of SDA (Sabouraud Dextro Agar) culture medium with 5 ml of the above concentration of the extract was poured in sterile petriplates and allowed to solidify. Then the test fungus was inoculated at the centre of the medium and incubated at room temperature 25 $^{o}C \pm 2\,^{o}C$. Replicates and controls were mainained through out the study. The diameter of the fungal growth was measured on 5^{th} and 7^{th} day.

To study the effects of antifungal alcoholic extract of above selected plants two sets of culture media were prepared separately for control and treatment. In the test sets of neutral pH 7, requisite amount of the experimental material were mixed and then added into the sterilized Sabouraud dextrose agar (SDA) medium of respective pH level. In the control set of each experimental set, the same volume of distill water (in place of experimental material) was mixed in appropriate amounts when ever found necessary.

Mycelia discs of 5 mm diameter, were cut from the periphery of 7 day old culture of the test organisms were aseptically inoculated upside down on the surface of the SDA medium in plaster. Inoculated petri plates were incubated at 25^{o} C $\pm$ 2^{o} C and observation were recorded at 5^{th} and 7^{th} day. Fungal growth taken as measurement parameter. The absence of Fungi denoted antifungal property of fungicidal nature.

Percentage of mycalial growth inhibition on different pH levels were calculated using following equation:

$$\%\text{growth inhibition} \quad \frac{\text{Colony diameter in control} - \text{colony diameter in treated sets x } 100}{\text{Colony diameter in control}}$$

$I = (C\text{-}t) \times 100$

Test fungi isolated and were used for in vitro studies. The culture was purified by hyphal tip technique. The stock culture of the test fungus was maintained on SDA medium at 25 degree C $\pm$ 1^{o} Degree C.

Effect of different concentration of aquous and alcoholic extract against the fungus was studied employing techniques.

Poisoned food technique. (Nene and Thapliyal, 1979) Poisoned food technique used to assess the antifungal activity of selected plant extracts. A series of double strength of test plant extracts viz. 10%, 25%, 50% and 75% were prepared using sterile distilled water. 30 ml of test extract was poured into 100 ml conical flask containing 30 ml sterilized melted SDA of double concentration. 30 ml of this mixed medium was then poured in each petriplate aseptically. The partiplates were inoculated with previously maintained 7 days old culture. 5 ml mycalial disc was cut with sterilized cork borer and transferred aseptically in the centre in inverted position. All petriplate including control and experimental were incubated at $25\,^{o}C \pm 1\,^{o}C$ for 7 days. After 7 days of incubation, observation were recorded and percent inhibition of radial growth was calculate using following formula:

$$I = \frac{C - T}{C} \times 100$$

where, I = percent inhibition
C = radial growth in check in mm/cm
T = radial growthin treated set in mm/cm

III. RESULTS

Table present investigations are being carried out to evaluate the antifungal medicinal properties of some higher plant against the pathogenic fungi viz., *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Microsporum fulvum.* antifungal activity of different concentration of alcoholic extracts of *Eucalyptus australe* plant parts on growth performances of *Aspergillus niger* given in table 4 and figure 6. Studies have shown that alcoholic plant part extracts of *Eucalyptus australe* are inhibitory to the growth of *Aspergillus niger*. Results have shown that inhibition of fungal growth increases with the increase in the concentrations of alcoholic extracts. Thus 10% alcoholic seed extract causes 20% inhibition of *Aspergillus niger* growth at 7^{th} day, however, this inhibition in 75% alcoholic seed extract at 7^{th} day is 76%.

Aspergillus niger

Table further shows that like seed root extracts of *Eucalyptus australe* also inhibits the growth of *Aspergillus niger* in culture medium. Thus, in 10%, 25% and 50% alcoholic root extract the growth is inhibited by 12%, 62% and 68% respectively at 7^{th} day as compared to control. Similarly Aspergillus niger growth is also inhibited by 19%, 63% and 68% respectively in alcoholic shoot extract concentrations of 10%, 25% and 50% respectively on 7^{th} day in comparison to control.

Aspergillus flavus,

Results of effects of various concentrations of alcoholic extracts of *Eucalyptus australe* plant parts on the radial growth of *Aspergillus flavus* given in Table 1. Growth studies done at 5th and 7th day, however, observation reveals that growth is more inhibited at 7th day in higher alcoholic extract concentration. Thus, at 7th day root, shoot and seed alcoholic extract inhibits growth of *Aspergillus flavus* by 50%, 51.7% and 50% of control respectively in 75% alcoholic extract concentration. Like wise, growth of *Aspergillus flavus* also inhibited in various other concentrations of alcoholic extract of this plant. Thus, growth of these fungi at 5th day in seed extract is 87.5%, 81.25% and 68.75% in 10%, 25% and 50% alcoholic concentration of seed respectively. Table also shows that like seed extract, root and shoot extract also shows inhibitory effect on growth of these fungi, however, inhibition increases with the increase in concentrations.

Candida albicans

Table 1 indicates the effect of alcoholic extract of *Eucalyptus australe* on the growth performances of opportunistic fungi, *Candida albicans.* Result shows that the growth of this fungi also inhibited by the alcoholic extract of various parts of *Eucalyptus australe*. Thus, radial growth values of these fungi 89.2%, 75%, 53.5% and 50% of control in 10%, 25%, 50% and 75% alcoholic root extract concentration respectively. Like wise, the growth of these fungi also affected in the presence of various alcoholic extract concentrations of shoot and seed. Result also shows that with the increase in plant extract concentration the rate of inhibition increases. Thus, in 10% alcoholic shoot extract the radial growth is 93.3% of the control where as same growth in 75% shoot extract is 50% of the control.

Microsporum fulvum

Table 1 shows the effect of different concentrations of alcoholic extracts of plant parts of *Eucalyptus australe* performances of *Microsporum fulvum.* Results have shown that growth of *Microsporum fulvum* is inhibited in the presence of various concentrations of root, shoot and seed alcoholic extracts mixed in SDA culture at both days of observations. Thus, in 10%, 25%, 50% and 75% alcoholic root extracts the growth of this fungi was 65%, 52.5%, 50% and 27.5% of control respectively in 7th day old culture plate. Similarly, the growth is inhibited in presence of alcoholic shoot and seed extracts in culture medium. However, the inhibition of growth found more at higher concentrations. So in presence of 75% alcoholic shoot and seed extract the growth of these fungi is 25.5% and 23.2% of control respectively at 7th day. Nearly similar pattern of growth inhibition found in various other concentrations.

IV. DISCUSSION

The present investigations are being carried out to evaluate the antifungal medicinal properties of *Carica papaya* plant against the pathogenic fungi viz. *Aspergillus niger, A.flavus, Candida albicans* and *Microsporum fulvum.* Fungal infections comprise an important faction of diseases occurring not only in plants and animals but also in human beings. Moulds and yeasts are so widely distributed in human environment that human beings are instantly exposed to them. Fortunately, because of the relative resistance of human beings and comparatively non pathogenic nature of fungi, most of these exposures do not lead to over infection. However, fungi are gaining importance with respect to increased incidence of chronic, often fatal, mycoses in immune compromised patients (De Hoog et. al., 2000). The fungi present in soil, water and air constitute exogenous fungal opportunists. The roster of opportunistic fungal species continues to increase. However, some of the common ones include *Aspergillus fumigates, A.niger, A.terreus, A.flavus, Absida, Candida albicans, Cryptococous neoformis, Microsporum fulvum, Mucor, Rhizomucor, Rhizopus,*and *Torulopsis globrata* (Singh, 1976). To find suitable drug for the management of fungal diseases is difficult because fungi, like human beings, are eukaryotes. Many of the cellular and molecular processes are similar, and still a number of chemicals are reported to have antifungal activity (Nene et al., 1979). These include the derivatives of quinazolinone. Farghaly and Moharram, 2000), coumarin (Hamkare et al., 2002), thiazolidinone (Datta et al., 2002), thiadiazole (Yu. et al., 2001), Thiazole (Jag, 2000), Pyridine (Bhatt et al., 2001) and Sydnone (Bekhit et al., 2002).

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Table – 1 effect of different concentration of alcoholic extracts of plant parts of *eucalyptus australe* on growth performance of *a. Niger, a. Flavus, c. Albicans* and *m. Fulvum.*

Days of Study	Diameter of Growth (Cm.) *A. Niger*			Diameter of Growth (Cm.) *A. Flavus*			Diameter of Growth (Cm.) *C. Albicans*			Diameter of Growth (Cm.) *M. Fulvum*		
	Root	**Shoot**	**Seed**	**Root**	**Shoot**	**Seed**	**Root**	**Shoot**	**Seed**	**Root**	**Shoot**	**Seed**
Growth in Control 0% extract												
5^{th}	3.0	3.2	2.7	1.9	2.1	1.6	1.5	1.6	1.7	3.0	3.1	3.0
7^{th}	5.0	5.3	5.0	2.8	2.9	3.0	2.8	3.0	2.9	4.0	4.3	4.3
Growth in 10% alcohalic extract												
5^{th}	2.5	2.6	2.0	1.7	2.0	1.4	1.4	1.5	1.6	2.0	2.6	2.7
7^{th}	4.4	4.3	4.0	2.5	2.8	2.6	2.5	2.8	2.7	2.6	3.1	3.1
Growth in 25% alcohalic extract												
5^{th}	1.3	1.4	1.2	1.5	1.8	1.3	1.2	1.1	1.2	1.6	2.2	2.4
7^{th}	1.9	2.0	2.0	2.3	2.1	2.2	2.1	2.5	2.3	2.1	2.6	2.7
Growth in 50% alcohalic extract												
5^{th}	1.0	1.3	1.2	1.2	1.1	1.1	0.9	0.8	1.0	1.4	1.2	1.7
7^{th}	1.6	1.7	1.6	1.8	1.9	1.7	1.5	1.6	1.9	2.0	1.4	2.0
Growth in 75% alcohalic extract												
5^{th}	0.8	0.9	0.7	0.8	0.9	1.0	0.8	0.6	0.8	1.0	1.0	0.7
7^{th}	1.3	1.2	1.2	1.4	1.5	1.5	1.4	1.5	1.2	1.1	1.1	1.0