SECTION 5

SUMMARY AND DISCUSSION
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The present investigation was undertaken to "Studies on Antifungal and Biochemical parameters of plant material with special reference to Dermatophytosis". During the present investigation antifungal potency of higher plants against Trichophyton mentagrophyte, Trichophyton rubrum, Microsporum gypseum and Microsporum nanum were studied both in-vitro and in-vivo in order to develop potent indigenous herbal therapeautant so that the various side effect found in synthetic drug could be over come. The findings of the present study are being discussed in the light of previous investigations.

Isolations of dermatophytes:

The isolation of dermatophytes were made from the O.P.D. section of dermatology department of M.L.B. Medical College, Jhansi under the supervision of Dr. Dinesh Govil. The isolates were brought to the lab for isolation & identification of the fungus on Sabouraud’s agar media. Out of 84 samples obtained 31 samples were of Trichophyton rubrum, one of Microsporum nanum, 40 of Trichophyton mentagrophyte, two of Microsporum gypseum and the rest samples were negative. This show that most of the patients were suffering from the infection of Trichophyton mentagrophyte followed by Trichophyton rubrum and then Microsporum gypseum while only one isolate was of
Microsporum nanum. Identification of isolates were confirmed on the basis of morphological and cultural characteristics consulting monographs and authentic cultures obtained from All India Institute of Medical Science, New Delhi.

Screening of plant for antifungal activity:

As described in the chapter “Introduction” large member of workers have screened higher plants against plant pathogens. However a systematic evaluation of higher plants against human pathogen remained comparatively neglected. In the present study 64 plants were screened which belong to twenty two families of Angiosperms. Out of these Cassia tora, Trachyspermum ammi, Ficus hispida and Vitex negundo showed maximum inhibitory effect against the four fungal organisms isolated from patients. The rest of the plant species exhibited varying degree of toxicity. Singh 1987 reported strong fungitoxic activity of the family Meliaceae.

Dixit & Tripathi 1975 found strong fungicidal toxicity in Caesalpinaceae. Giliver 1947; Dixit 1978; Singh et al., 1986 found strong fungicidal activity in Umbelliferae. Kishor et al., 1981, found Verbenaceae to be actively fungicidal. These results are in conformity with the results of the authors. Considering the findings of the previous workers that plant parts differ in fungitoxicity. Stwarts and Medrik 1968 found corn berry juice fungitoxic against dermatophyte. Ahmed et al., 1977 found Juglans regia bark fungitoxic against Microsporum gypseum;
Tansey and Appleton 1975, found bulbs of Allium sativum active against Microsporum gypseum and Trichophyton rubrum. Mukharya and Dahia 1977, found root of Plumbago species active against Microsporum gypseum. Chile et. al., 1981 found the entire plant of Vinca rosea active against Trichophyton rubrum in which the leaves showed maximum activity. Joshi and Bhatt 1983, found Chakramad B. durva active against Microsporum and Trichophyton species. Similarly many worker like Singh 1984; Rao & Rao 1985; Tripathi et. al., 1988; Gupta 1988; Mishra et. al., 1988; Tripathi et. al., 1990 etc. have found different parts or different plants, fungicidal against dermatophytes. The authors have found the positive activity of some of these plant but few of these showed significant activity and therefore were selected for further study. These results of the author are therefore in conformity to the result of the previous work. Various workers like Mishra have observed the fungitoxicity varied from genus to genus within a family as 1975; Tripathi 1980; Asthana 1984. Variations in fungitoxicity were also observed from species to species within the same genus. Out of the two species of genus Cassia only Cassia tora exhibited strong toxicity while the other showed poor toxicity. Thus plants containing fungitoxicity are scattered throughout the flowering plants and their activity is quite unrelated to their taxonomic position. Even within the same plant, some parts are more fungitoxic than the other part of the same plant. The leaves of the most of the plants were found to be more toxic than the other parts. In the present study, leaves of different plants were used during the preliminary screening later other parts were
studied. Since in the present study airdried parts were found to lose their antifungal activity thus the fresh part of the plants were used in the present study. This observation is inconformity to those of Gilliver 1947; Abdulla, 1959; Dubey 1981 and Asthana, 1984.

In the present study four plants out of 64 originally screened were selected to study the presence of fungitoxicity in different part of these plants. These plants were *Cassia tora*, *Vitex negundo*, *Ficus hispida* and *Trachyspermum ammi*. It was observed that pods of *Cassia tora*, leaves of *Vitex negundo* and *Ficus hispida*, while seeds of *Trachyspermum ammi* showed better fungitoxicity as compared to their other parts. From this experiment it was also observed that the above part of *Cassia tora*, *Vitex negundo* and *Ficus hispida* gave better results than *Trachyspermum ammi*. Therefore three parts of the above plants were selected for further study.

Extracts of plant parts were employed for antifungal tests in the present study. For this water extracts and organic solvent extracts were used. Singh 1980; Dubey et. al., 1982; Pandey et. al., 1983; Asthana 1984; Chandra 1984; Mall 1987 and Gupta 1988 also used water extracts. while different organic solvents were employed by others like Tripathi, 1976, 1977; Chaturvedi, 1979; Dixit, 1980; Saxena 1980. In the present study aqueous extracts of selected parts of the plants were preferred for antifungal screening. Since water being the most polar solvent it facilitates the extractions of maximum constituents from the plant’s part.
The water extract obtained from *Cassia tora* pods *Vitex negundo* and *Ficus hispida* leaves were mixed with sabouraud’s agar media and their effect on the radial growth of the four test organism were studied. Radial growth of *Trichophyton mentagrophyte*, *Microsporum Gypseum* and *Microsporum nanum* were measured after every 24 hours while that of *Trichophyton rubrum* were measured after every 48 hours, because of the very slow growth of later fungus. *Trichophyton mentagrophyte* was found to be best inhibited by the immature pods extract of *Cassia tora*. *Microsporum gypseum* *Microsporum nanum* and *Trichophyton rubrum* were best inhibited by *Ficus hispida* leaves extract.

When the inhibitory effect was measured in terms of percentage inhibition a slightly different picture developed. *Cassia tora* water extract had highest percent inhibition against *Trichophyton mentagrophyte* followed by *Trichophyton rubrum*. *Microsporum nanum* and *Microsporum gypseum*. The inhibitory effect of *Vitex negundo* leaf extract was also found in the same sequence. On *Ficus hispida* leaf extract *Microsporum nanum* showed best percentage inhibition followed by *Trichophyton mentagrophyte*, *Trichophyton rubrum* and *Microsporum gypseum* respectively.

**Antifungal study of Plants solvent extract:**

Various workers have used organic solvents for extraction of fungitoxic constituents of higher plants. Doskotch et al., 1975; Turner et al., 1975; Dixit et al., 1976; Tripathi et al., 1978 used solvents. For this number of solvents are available for extraction of
different chemicals present in the plants. In the present study extracts from active plant parts were made in acetone and methanol solvents using Soxhlet apparatus. These were tested against the four test organism namely *Trichophyton mentagrophyte*, *Trichophyton rubrum*, *Microsporum gypseum* and *Microsporum nanum* respectively, using glass cylinder fixed on fungus seeded Sabouraud Agar media. The zone of inhibition obtained was noted.

From the results obtained it was found that acetone extract was better inhibitory as compared to methanol extract. The two species of *Trichophyton* that is *Trichophyton mentagrophyte* and *Trichophyton rubrum* were better inhibited by *Cassia tora* pod extract, while the two species of *Microsporum* that is *Microsporum gypseum* and *Microsporum nanum* were best inhibited by *Ficus hispida* leaf extract. *Vitex negundo* leaf extract was generally found to be moderately effective.

**Antifungal study of oils extracted from active plant parts:**

Extraction of fungitoxic constituent by hydrodistillation technique has also been adopted by various workers, such as Chaturvedi 1979; Grover and Rao 1979; Asthana et. al. 1982; Renu et. al., 1985. In the present study hydrodistillation was conducted by Perkin’s apparatus, the extracted oils were used with acetone so that it could freely diffuse in agar medium. These oil samples were placed in glass cylinder fixed on test fungus seeded agar. Sabouraud’s medium. The inhibitory zones developed were studied after 14 and 21 days. From this study it was found that *Cassia tora* oil produced best inhibitory effect on
Trichophyton mentagrophyte followed by that of Cassia tora and Vitex negundo. Microsporum nanum were best inhibited by Ficus hispida while Cassia tora and Vitex negundo oils were next in sequenc. Trichophyton rubrum was best inhibited by Cassia tora oil followed by Vitex negundo and Ficus hispida. Microsporum gypseum was best inhibited by Ficus hispida oil followed by that of Ficus hispida and Vitex negundo. These observations confirm above previous result obtained with solvent extract.

Previously many workers have used oils obtained from plants for their antifungal activity. Asthana, 1984; Kishore 1985; Mall 1987 isolated oils from active plants while others like Sharma & Singh 1979 tested the commercial oil for their fungitoxicity. These workers have not paid much attention on the detail fungitoxic property such as its possible therapeutant use. In the present investigation oils were subjected to detail fungitoxic study. Such as minimum inhibitory concentration, fungicidal or fungislastic nature of oil, it's sensivity on human skin and efficiency of the oil for curing infections, there effect on biochemical parameters of blood in albino rats.

**Antifungal activity of composite sample of selected plant material:**

This experiment was conducted with a view to get a composite samples in which samples from all the three plants could be
mixed so that it could be used for controlling infections caused by any of these fungus.

In this experiment acetone solvent extract obtained from active plant part of three plants Cassia tora, Vitex negundo and Ficus hispida were mixed in different proportions. In all, four such composite sample were prepared. One in which all the three plant material were mixed in equal proportion and the other three in which one of the plant material was reduced to half the quantity of other two plant materials.

From this experiment it was observed that samples which had Cassia tora solvent extract in dominating position gave better result against Trichophyton mentagrophyte and Trichophyton rubrum when Ficus hispida extract dominated it gave better result against Microsporum gypseum and Microsporum nanum.

Antifungal activity of solvent extract on spore germination:

In this experiment inhibitory effect of solvent extract on spore germination of the four fungal organisms were studied. From this experiment it was found that Trichophyton mentagrophyte spores were 80% inhibited when treated with Cassia tora extract, 60% with Vitex negundo extract and 50% with Ficus hispida extract. Microsporum gypseum spores germinations were inhibited to 32%, 30% and 47% with extract of Cassia tora, Vitex negundo and Ficus hispida respectively. Microsporum nanum spores were 70%, 55% and 50% inhibited with Ficus
hispida, Cassia tora and Vitex negundo respectively. While Trichophyton rubrum spores were 70% inhibited with Cassia tora extract, 60% with Ficus hispide and 50% with Vitex negundo. From these data and those obtained on radial growth it appears that these plant extract inhibited both spore germination and fungal hyphae development.

Minimum inhibitory concentration:

This experiment is important specially when minimum doze of the fungitoxic substances is to be recommended when used for in-vivo test. Overdosing may result in wastage of the fungitoxic substance or may impart toxicity to the host.

For this five different concentrations of the oil were prepared in acetone. these were 5000, 2500, 1250, 625 and 312 ppm. of each oil. The inhibitory zones obtained measured after 14 days of incubation, it was observed that inhibitory zone started from 1200 ppm concentration therefore this concentration was regarded as minimum inhibitory concentration of the oil.

Several persons have tried to determine the MIC of various essential oils. Pandey, et. al., 1983 has found Ageratum haustonianum oil to have 100 ppm MIC concentration against Microsporum gypseum. Singh et. al., 1986 has found 900 ppm concentration of Trachyspermum ammi against Trichophyton mentagrophyte. In 1987 they found 1000 ppm concentration of Hyptis suaveolans to be MIC against the same fungus in 1983,
they found 1000 ppm concentration of *Ocimum gratissium* to be MIC against the same fungus.

The variation seems to be due to different sensitivity of particular oil against the test fungus it may also be due to different technique used during antifungal investigations. The MIC concentration obtained in the present in the present investigation is almost conformity to the above observations. The slight difference may be due to the nature of oil and sensitivity of the test organism towards the oil tested.

**Fungistatic or fungicidal nature of oil:**

The substances toxic to the fungi may inhibit the growth of fungi either temporarily or permanently. When fungi is temporarily inhibited the fungitoxic substances are regarded as fungistatic and when fungi is permanently inhibited the fungitoxic substances are fungicidal. Oils of *Ageratum houstoniaum* Pandey et al., 1983; *Ocimum Canum* Dubey, 1981. *Cympogon martini* Singh et al., 1980 showed fungistatic nature of the oils. While *Hyptis suaveolans* Pandey et al., 1982. *Chenopodium ambrosioides* Kishore, 1985 exhibited fungicidal nature of the oil at MIC concentrations.

In the present study it was observed that all the three oils, obtained from *Cassia tora*, *Vitex negundo* and *Ficus hispida* were fungicidal at 2500 and 5000 ppm concentration against all the four fungal organisms. At 1250 ppm concentration all the
above three oils were fungicidal against *Trichophyton mentagrophyte* but fungistatic against *Trichophyton rubrum Microsporum gypseum* and *Microsporum nanum*. This shows that the oils showed fungistatic nature at their MIC concentration but at higher concentration they exhibited fungicidal nature. Thus their fungicidal or fungistatic nature is dose dependent.

**Sensitivity test obtained from active plants material on human skin:**

Any therapeutic agent before being prescribed for treatment must be tested both in-vitro and in-vivo conditions to prove as a safe agent for the control of disease since detailed in-vitro study on essential oils of *Cassia tora, Vitex negundo* and *Ficus hispida*, has indicated their potentiality as their ideal antifungal agent against dermatophytes. These were than subjected for in-vivo investigation so as to confirmed their efficiency as therapeutant for dermatophytic diseases. Most of the earlier investigation was confirmed on in-vitro studies only.

In the present study in-vitro investigations were made on human skin and the first preference the sensitivity of the oil was tested on human skin after 10 days of their application. It was observed that none of the oil produced any allergic response when applied to different persons. Out of 25 persons to whom *Cassia tora* oil was applied only one complained of mild irritation, one has soothing effect and the rest 23 had no effect. In *Vitex negundo*
oil ointment out of 25 persons, 22 had no effect, one showed irritation while the rest two had soothing effect.

_Ficus hispida_ oil, out of 25 persons, 2 had mild irritation, 3 had soothing effect and rest 20 had no effect. This shows that these oil could be safely used on human skin without any allergic responses.

**Efficiency of the oil for cure of infection:**

For in-vivo study mostly experimental animals were employed. Kligman, 1956 induced experimental ringworm infection to mice. Nandi & Bose, 1976 however suggested that these animals have short duration of susceptibility to the disease and therefore they are unsuitable as test animal for in-vivo trials. Others have used guinea pigs for evaluation of topical antifungal drugs.

In the present investigation observation have been made in the form of percent culture recovery (Wahab et.al., 1982) with the application of the oil. The oil used in the present study was in the form of ointment made in petroleum jelly, which has quick penetration in the skin. Since ointment has animal vegetable, or mineral greases they are more suitable then non-greasy substances. Oil also increased the pliability of dry skin and work as vehicle for penetration of the drugs in skin. As such in the present study the petroleum jelly has been used as base for the preparation of the ointment. In the present study no experimental infection were made on human skin. Only already infected
patients were used for the study, after confirmation for the establishment of infection by particular fungus both through direct examination and culture positive test. Direct examination was made through collection of samples from infected area, their direct examination with 10% KOH solution under the microscope and later by raising their culture on Sabouraud’s agar medium. After confirmation In-vivo studies were made by applying the ointment made up with oils and their application twice a day. The results were recorded in terms of percent culture recovery on alternate days from infected area.

During this study infections caused by *Trichophyton mentagrophyte*, *Trichophyton rubrum* and *Microsporum gypseum* were treated since during in-vivo test no patient suffering from *Microsporum nanum* could be found. For in-vivo test *Cassia tora* oil and *Ficus hispida* oil were used since these gave better result during in-vitro test as compare to *Vitex negundo* oil.

In this study complete recovery from *Trichophyton mentagrophyte* infection was obtained after 21 days treatment with *Cassia tora* oil ointment and 23 days treatment with *Ficus hispida* oil ointment. *Trichophyton rubrum* infection was completely recovered after 23 days treatment of *Cassia tora* oil ointment and more then 23 days treatment with *Ficus hispida* oil ointment. *Microsporum gypseum* was completely cured after 21 days treatment of *Ficus hispida* and 23 days treatment of *Cassia tora* oil. This clearly show that *Trichophyton* could be better cured with *Cassia tora* oil ointment while *Microsporum* could be
better cured by *Ficus hispida* oil ointment. Thus the present investigation suggest that essential oils of *Cassia tora* pods and *Ficus hispida* leaves may prove to be promising therapeutant for the cure of dermatomycosis caused by these organisms.

**Effect of plant material on the Bio–chemical parameters of blood in Albino rats:**

Before effective use of any therapeutant it is necessary to find it’s effect when it comes in contact of blood. Since external substances bring changes in the physiology and biochemical distribution within the blood. It also helps in designing the future strategy in drug management. Since rats are used in most of the biological studies and they are easy to be maintained and experimented therefore the effect of the oil on blood were studied on rats. During the past none of the investigator studying on fungitoxic substance has investigated, it’s effect on blood, which is very essential while recommending the safe use of a therapeutic agent.

The present study was made on behaviour, food consumption, body weight, total erythrocyte count, total leucocyte count, Hemoglobin content, Blood glucose and Serum cholesterol in *Rattus norvegicus*. It was observed that the experimental animal behaved normally without any exudation of Saliva from mouth hand legs or body tremors immovability, prostation etc. upto 21 days of treatment. The food consumption of the experimental animals remains normal during the treatment. Although a slightly
higher consumption of food was found in controlled rat. Since the difference is not much therefore it is quite insignificant.

The body weight of the controlled and treated rat also remains the same, which suggest that difference in food consumption may be temporarily without any significance. When the total erythrocyte count of the controlled rat was compared with that of the treated rat it was found that neither Cassia tora ointment nor Ficus hispida ointment had any ulterior effect on the total erythorocyte count. The total leucocyte count showed a slightly increased in the treated rat as compare to control rat. Which might be due to the injury to rat while applying the ointment. The data obtained on hemoglobin-content of the rat showed both the ointment had not adversely affected, the hemoglobin content of the blood in rats. They maintained almost the same level. Similarly there was no effect on the blood glucose or serum cholesterol from either of the ointment. They were maintained almost in the same level as found in the control.

Thus from the over all findings of the present investigations it can be said that the oils of Cassia tora and Ficus hispida appear to be very positive and promising for the treatment of dermatomycosis caused by Trichophyton mentagrophyte, Trichophyton rubrum and Microsporum gypseum on account of the fact that they have strong fungicidal activity without any irritating or burning effect on the human skin. They have short killing time and have no physiological or biochemical effect in the composition of blood when they come in the direct contact of the blood.