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DETERMINATION OF ANTIFUNGAL ACTIVITY OF *CEDRUS DEODARA* ROOT OIL AND ITS COMPOUNDS AGAINST *CANDIDA ALBICANS* AND *ASPERGILLUS FUMIGATUS*

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Abstract

Studies have carried out to investigate the antifungal activity of *Cedrus deodara* root oil and compounds of this oil against *Candida albicans* and *Aspergillus fumigatus. Cedrus deodara* oil at the concentration of 150 µg/disc showed zone of inhibition against *A. fumigatus* but at the same concentration did not show any antifungal activity against *C. albicans*. Similarly compounds of *C. deodara* oil such as trans-atlantone and allo-himachalol also have not shown any antifungal activity, while himachalol at the concentration of 150 µg/disc showed zone of inhibition against *A. fumigatus*.

Introduction

Cedrus (true cedars) a very important genus of trees belonging to Pinaceae, has a selected distribution in the Mediterranean region. In Pakistan it is found in the hilly areas of Northern Pakistan. Its evolution and biogeography are of great interest to botanists. It is an ever-green tree with spreading horizontal branches having small leaves. It has many terpenoids. Previously in the Indus Unic system various parts, extracts and oils of this plant have been used frequently on patients for the treatment of different diseases. For instance, some local medical personnels i.e., Hakims claim that its wood extract is carminative, diaphoretic and useful in fever, flatulence, pulmonary and urinary distention, rheumatism, piles and kidney stones. Also its bark extract is astringent and useful for fever, diarrhoea and dysentery as reported by Baquar (1989). Its root extract or oil is also diaphoretic containing mostly terpenoids such as Himachalol, Atlantone and Trans-atlantone, which are same that are found in trunk oil (Bisarya & Devis, 1968a; 1968b; Pande *et al*., 1971). They also claim that the root oil of this plant is very effective in the treatment of fungal infections and used as antifungal agent in this region. Tandan *et al*., (1989) reported that 15% of *Cedrus deodara* in castor oil (i.e., formulation of 15 ml *Cedrus deodara* root oil and 85 ml Castor oil) was useful as a dermal application to control fungal infection in rabbits when exposed daily for 21 days. They also reported that no significant changes occur in their body weights, organ weight and organ / body weight ratio in the formulation treated animals.

Chowdhry *et al*., (1997) reported that one of the important compounds of the *Cedrus deodara* root oil named himachalol has antifungal activity. Cheng *et al*., (2005) investigated that the chemical composition and antifungal activities of essential oils from different tissues of Japanese Cedar (*Cryptomeria japonica*) against wood decay fungi and tree pathogenic fungi and they reported that the essential oil obtained from Japanese Cedar Heartwood showed excellent antifungal activities against *Laetiporus sulphureus*,

Trametes versicolor, *Rhizoctonia solani*, *Collectotrichum gloeosporioides*, *Fusarium solani* and *Ganoderma australe* as compared to the essential oils from other tissues. Very few studies have been conducted on root oil of *Cedrus deodara* related to its antifungal activity, however some work has been done on *Cedrus deodara* oil but on different line of assessment such as enzyme activities in the nervous tissue of terrestrial snails, cell pathology, prevalence of allergic response by pollen allergens, cytotoxic activity during *in vitro* screening of medicinal plant extracts (Rao *et al*., 2003; Bist *et al*., 2005; Shashi *et al*., 2006; Nisha *et al*., 2007). Perveen *et al*., (2008) reported the mammalian toxicity of *Cedrus deodara* root oil against Albino rats (Wistar strain). They reported 34.4gm/kg as LD_{50}, which was quite safe as compared to neem oil LD_{50}, 5gm/kg. They also reported GC-Mass analysis and spectral studies of this oil. This oil has also been reported as anti-ulcer agent by Hakeems, being used orally. Tariq *et al*., (2010) reported anti-fungal activity of *Acorus calamus* oil, tested for cuts and wounds on sheep in Hub-Balochistan. The *A. calamus* oil prevents the cuts and wounds from fungal growth and the cuts and wounds heal rapidly as compared to control. Since very little work has been done in relation to antifungal, anti-ulcer and anti-inflammatory effects of *Cedrus deodara* in India, Pakistan and other countries therefore, the present study has been conducted to assess these effects.

Materials and Methods

To determine the *In vitro* antifungal activity of *Cedrus deodara,* extract of neutral fraction, acidic fraction, pet-ether soluble fraction and pure component of *Cedrus deodara* including himachalol, trans-atlantone, allohimachalol have been used. A modified method of Ahmad *et al*., (1984) was adopted to test the antifungal activity. For this sterilized thick filter paper disc (5 mm diameter) were loaded with each dilution of 5 mg / ml and kept in sterile condition until used. Disc of control, positive control and loaded discs were placed at different positions in the Petri dishes. Nystatin was used as positive control. Regarding antifungal activity, Petri plate containing potato dextro agar medium were seeded with suspension of *Aspergillus fumigatus* and *Candila albicans* before placing the disc (10 µg/disc). Plates were incubated at 28°C for 3-5 days and zone of inhibition were recorded.

Result and Discussions

In the present study *Cedrus deodara* root oil and its compounds were used for determining antifungal activity against two important fungi i.e., *Aspergillus fumigatus* and *Candida albicans* as compared with negative control and positive control (Nystatin). *Cedrus deodara* oil in concentration of 150 µg / disc and pure form showed 7 mm and 8 mm zone of inhibition respectively against *Aspergillus fumigatus*. *Cedrus deodara* oil did not show any antifungal activity against *Candida albicans* at the same concentration.

Acidic fraction and petroleum fraction of *Cedrus deodara* root oil as well as constituents of *Cedrus deodara* oil such as trans-atlantone and allo-himachalol showed no antifungal activity against *Candida albicans* and *Aspergillus fumigatus* at any concentration (µg/disc). Himachalol is another constituent of *Cedrus deodara* oil which showed antifungal activity with the zone of inhibition of 10 mm at the concentration of 150 µg/disc against *Aspergillus fumigatus*.

Table 1. Antifungal activity of *Cedrus deodara* root oil and its compounds against *Candida albicans* and *Aspergillus fumigatus.*

S. No.	Cedrus deodara oil/components of *Cedrus deodara oil*	Concentration (µg/disc)	Zone of inhibition (mm)	
			Candida albicans (CA)	*Aspergillus fumigatus (AF)*
(i)	*Cedrus deodara oil*	30	-	-
		60	-	-
		90	-	-
		120	-	-
		150	-	7 mm
		Pure	-	8 mm
(ii)	Acidic fraction	30	-	-
		60	-	-
		90	-	-
		120	-	-
		150	-	-
		Pure	-	-
(iii)	Petroleum ether fraction	30	-	-
		60	-	-
		90	-	-
		120	-	-
		150	-	-
		Pure	-	-
(iv)	Himachalol	30	-	-
		60	-	-
		90	-	-
		120	-	-
		150	-	10 mm
(v)	Trans-atlantone	30	-	-
		60	-	-
		90	-	-
		120	-	-
		150	-	-
(vi)	Allo-himachalol	30	-	-
		60	-	-
		90	-	-
		120	-	-
		150	-	-

Himachalol did not show any antifungal effect at any concentrations (µg/disc) against *Candida albicans.* The *In vitro* test for antifungal activity of crude oil, himachalol and allohimachalol with zone of inhibition can also be seen in Fig. 1.

Chawdhry *et al*., (1997) reported about the antifungal activity of himachalol against *Aspergillus fumigatus*. The present study also confirms the above investigations. Talwar *et al*., (1997) reported the antifungal activity of neem oil and the zone of inhibition was found against *Candida albicans* and *Candida tropocalis*. In the present study antifungal activity of *Cedrus deodara* oil and its constituents were noted with the zone of inhibition against *Aspergillus fumigatus*, which confirms the above report.

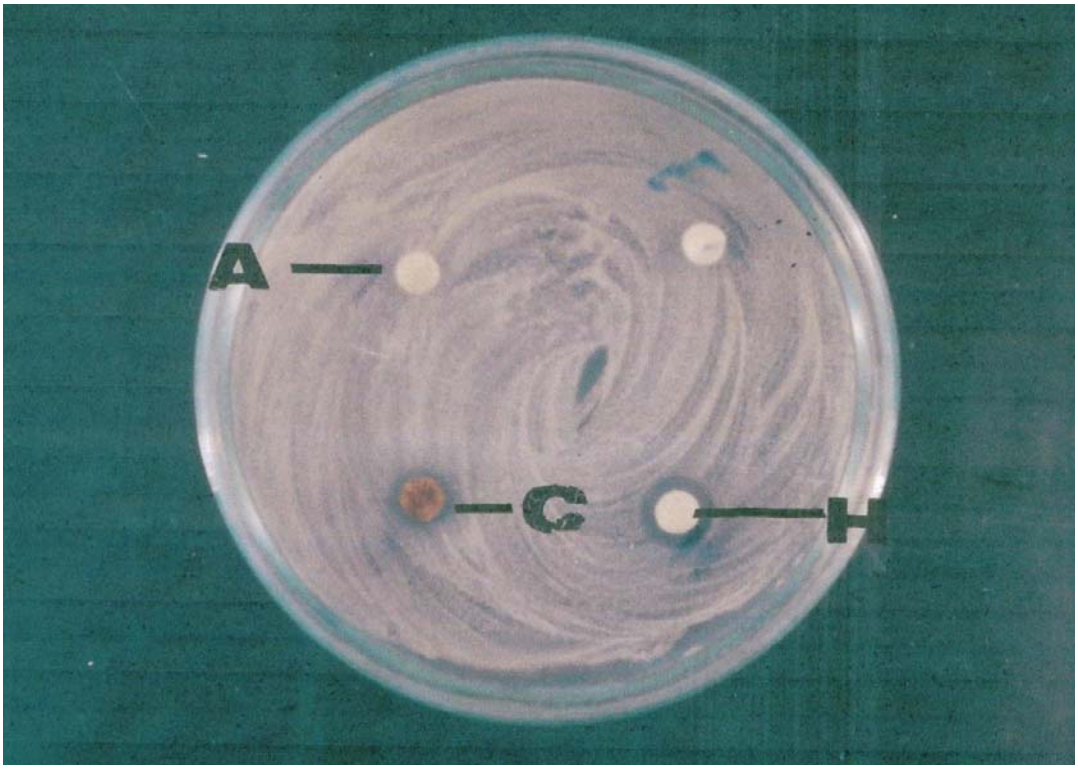

Fig. 1. Photograph of *In vitro* test for antifungal activity of crude oil (C), Himachalol (H) and allo-himachalol (A), zone of inhibition can be seen in the case of crude oil (8 mm) and Himachalol (10 mm).

Bhonde *et al*., (1999) reported the control of *Fusarium oxysporum, Alternaria solani* under *In vitro* condition by neem oil preparation. The present study is concerned with antifungal activity under *In vitro* condition by the *Cedrus deodara* oil and their constituents and showed zones of inhibition against *Aspergillus fumigatus*.

Fandohan *et al*., (2004) reported *In vitro* and *In vivo* inhibition of *Fusarium verticillioides* by some essential oils. They also reported that control of fungi at concentrations of 4.8, 6.4 and 8.0 µ/g respectively. The present study confirms the earlier reports.

Conclusion

Antifungal activity of *Cedrus deodara* oil at the concentration of 150 µg/disc was determined against *Candida albicans* and *Aspergillus fumigatus.* The experiment showed zone of inhibition against *Aspergillus fumigatus* but did not show any response regarding antifungal activity against *Candida albicans.* Two compounds i.e., Trans-atlantone and Allo-himachalol of *Cedrus deodara* oil did not show any antifungal activity whereas zone of inhibition was seen by himachalol at the concentration of 150 µg/disc against *Aspergillus fumigatus.*

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