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***In vitro* antifungal activity of ethanolic extract of *Cocculus laurifolius* leaves**Sidra Maqbool^{1*} & Ishrat Younus¹

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Abstract: The present study was designed to investigate the antifungal activity of ethanolic extract of *Cocculus laurifolius* leaves. The concentrations of 100, 200 and 400 µg/ml of ethanolic extract were used to evaluate the antifungal potential of extract. The experiment was conducted using agar disc diffusion method against five different fungal strains: *Aspergillus niger*, *Micro sporumcanis*, *Candida glabrata*, *Trichophyton rubrum* and *Candida albicans*. The antifungal activity was observed by estimating the zone of fungal growth inhibition. *Cocculus laurifolius* showed significant decrease in fungal growth at all selected concentrations. However, the extract revealed maximum zone of inhibition at higher doses. The outcome of this study illustrated that ethanolic extract of *Cocculus laurifolius* leaves possess substantial *in-vitro* antifungal properties.

Keywords: *Cocculus laurifolius*, ethanolic extract, agar disc method, *in-vitro* antifungal activity

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INTRODUCTION

Globally fungal infections have been considered as major health related problem (1). Each year many billions of people suffer from fungal infections and reportedly 1.6 million patients died due to severity of these infections (2). Despite the availability of multiple antifungal agents, researchers are focused to develop safe and effective treatment options. Recent developments in phyto-medicines also focused to investigate novel treatment protocols against different fungal strains to reduce the burden of fungal infection. Previously, different plant species have been reported to possess antifungal properties (3).

The plant *Cocculus laurifolius* belongs to the family Menispermaceae (also known as moonseed). Two of the species of this plant have been reported in Pakistan (4). The

plant contains rich amount of alkaloids, flavanoids, saponins, tanins and phenolic components (5). The leaves extract of the plant have been previously reported for its neuromuscular blocking, anti-hypotensive (6), anticonvulsant, anxiolytic, hypnotic (7), anti-inflammatory and antimicrobial activity (5). Bark and leaves extract of plant have also been reported to possess *in-vitro* antifungal effects. Considering the above reported studies, the present study was designed to evaluate the antifungal potential of ethanolic extract of *Cocculus laurifolius* leaves by using agar disc diffusion method.

MATERIALS & METHODS**Collection of plant material**

Fresh leaves of *Cocculus laurifolius* were procured and identified from herbarium of G.C University Lahore, Pakistan.

**Table 1: Antifungal activity of *Cocculus laurifolius* leaves extract and Miconazole (standard drug) in different fungal strains**

Drug substance	Doses	Zone of Inhibition (mm)				
		<i>Aspergillus niger</i> ,	<i>Microsporu mcanis</i>	<i>Candida glabrata</i>	<i>Trichphyton rubrum</i>	<i>Candida albicans</i>
Ethanolic extract of <i>Cocculus laurifolius</i>	100 µg/ml	15	13	16	14	15
	200 µg/ml	19	18	17	16	20
	400 µg/ml	20	22	19	20	24
Standard drug Miconazole	100 µg/ml	16	18	15	18	19
	200 µg/ml	20	22	22	21	23
	400 µg/ml	25	28	27	29	26

Data expressed as Mean \pm SD, $p < 0.05$

Extraction of plant material

The leaves were washed thoroughly to remove debris and contamination. After thorough washing, leaves were dried under shade and ground to coarse powder which was then macerated in 98% ethanol for 7 days. The solvent was filtered and concentrated with the help of rotary evaporator at 45°C to obtain the concentrated extract. The percentage yield of extract was calculated and stored in air tight container at 4°C.

In vitro antifungal activity

1. Preparation of Media and doses

Five different fungal species have been selected for the evaluation of *in-vitro* antifungal activity; *Aspergillus niger*, *Microsporum canis*, *Candida glabrata*, *Trichphyton rubrum* and *Candida albicans*. The fungal media were prepared in Sabouraud dextrose and PDA, maintained at 4°C for fungal growth. For dose preparation of test

group, the ethanolic extract of *Cocculus laurifolius* was initially dissolved in DMSO, sterilized filtered (sintered glass filter) and stored at 4°C whereas commercially available Miconazole was used as a standard drug.

2. Agar disc diffusion method

In-vitro antifungal activity of *Cocculus laurifolius* leaves against five different species of fungi was conducted by using agar disc diffusion test (8). Three different concentration of ethanolic extract and standard drug Miconazole (100, 200 and 400 µg/ml) were prepared using nutrient agar tubes in double distilled water. The zone of antifungal inhibition was determined by measuring the size of disc at 28°C after 1 week. The triplicate experimental results in ethanol were assessed which did not produce any inhibitory effect on all test concentrations.

STATISTICAL ANALYSIS

All data was analyzed by using one way ANOVA (Analysis of variance) \pm SD and a



probability value of $p < 0.05$ was considered as significant. Statistical Package for the Social Sciences (SPSS) version 20 was employed for data analysis.

RESULTS & DISCUSSION

In current study three different concentrations of ethanolic extract of *Cocculus laurifolius* leaves were evaluated for the *in-vitro* antifungal activity against five different fungal strains. Results obtained from the study demonstrated that the extract significantly inhibited fungal growth at all selected concentrations. It is also noted that with the increase in concentration the zone of growth inhibition were also increased. The zone of inhibition for different fungal strains lie between 13-24 mm and 15-29 mm for ethanolic extract of *Cocculus laurifolius* and miconazole respectively (Table 1). The antifungal effects of leaves extract and miconazole have been illustrated in (Table 1). Plants have been traditionally used for their variable constituents and multiple therapeutic properties. In recent years many plants have been evaluated for their antimicrobial properties against different fungal species (9). These phytochemical substances can decrease pathogens growth by providing minimum damage to the host cells and are considered as a suitable option in the development of antifungal agents (10). Similarly, plants of *Cocculus* species have also been reported for antifungal potential. *Cocculus hirsutus* demonstrated antifungal effect against *Aspergillus flavus* (11). Similarly, *Cocculus laurifolius* have also been reported for its antifungal properties against *A.niger* and *F. solani* strains. In 2003, Srinivas demonstrated that antifungal activity of plants may be

contributed by the presence of flavanoids, so it can be assumed that the flavanoids of *Cocculus laurifolius* might be responsible for its antifungal effect (12). However, some researchers emphasized the involvement of saponins and tanins for antifungal properties of plant (13, 14). Moreover, the isoquinoline saporphine benzyl isoquinolines and Erythrina alkaloids might be responsible for antifungal activity of *Cocculus laurifolius*, as different plant alkaloids of solanum (15), *Chimonanthus praecox* (16) and *Zanthoxylum nitidum* (17) have been reported to produce antifungal effect. So, it can be assumed that the occurrence of these compounds in *Cocculus laurifolius* might be responsible for its antifungal activity.

CONCLUSION

In the present study, ethanolic extract of *Cocculus laurifolius* leaves showed noteworthy *in-vitro* antifungal activity. However, further research is needed to elucidate the therapeutic potential of this plant in different fungal strains and to evaluate the specific mode of action responsible to produce antifungal effect.

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