

Effect of Crude Extracts of *Apama Siliquosa* against Fungal Pathogens

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Abstract:- *Apama siliquosa* Lamk is an important medicinal plant whose leaves and roots have multiple uses. The plant is a rich source of many bioactive compounds which are of medicinal importance. The study was undertaken to investigate the antifungal activity of the methanol, chloroform, ethyl acetate and hot water extracts of the roots of *Apama siliquosa*. The extracts were tested against the pathogenic fungi *Candida albicans*, *Aspergillus niger*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Microsporum gypseum*. The extracts showed significant inhibitory activity against some fungal pathogens

Keywords:- *Apama siliquosa*; Antifungal activity; crude extracts

I. INTRODUCTION

Antibiotic resistance has become a global concern^[1]. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientists to search for new antimicrobial substances from various sources like the medicinal plants. World plant biodiversity is the largest source of herbal medicine and still about 60 to 80 percent of world population rely on plant based medicines^[2]. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes^[3]. Traditional healing systems around the world that utilise herbal remedies are an important source for the discovery of new antibiotics^[4]. This necessitates the need for further research into traditional health systems.

Apama siliquosa is a shrub found in evergreen forests of Western Ghats from Konkan to Kerala. An erect medicinal plant with smooth yellowish grey bark, alternate, aromatic leaves purple or greenish flowers in clusters. The roots are used as medicine in the treatment of many diseases like diarrhoea, dysentery, cholera, ulcers. The whole plant of *Apama siliquosa* Lamk is used for snake treatment^[5].

II. MATERIALS AND METHODS

A. Preparation of Extracts

A. siliquosa was collected from Alibaugh, Maharashtra, India. For the preparation of hot water extracts, ten grams of the dried root was left in distilled water for 6 hours at slow heat (35°C). After 12 hours, it was filtered through Whatman No.1 filter paper and centrifuged (Remi) at 5000 g for 15 minutes. The supernatant was collected and concentrated to make the final volume, one fifth of the original volume.

For solvent extraction, ten grams of the dried plant material was extracted with 100 ml of solvent (methanol, ethyl acetate, chloroform) and was kept on a magnetic stirrer (Remi) for 24 hours. Thereafter, it was filtered and centrifuged at 5000 g for 15 minutes. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume^[6].

B. Anti fungal activity

The extracts of *A. siliquosa* was tested against the fungi: *Candida albicans*, *Aspergillus niger*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Microsporum gypseum*

Fungal cultures were subcultured in Sabourad dextrose Agar (SDA) and diluted to obtain a final organism density of 5×10^5 CFU/ml. The test was performed in 96-well sterile microplates. All the wells received 100 μ l Sabourad broth (for fungus) supplemented with 10% glucose and 0.5% phenol red. An adaptation of the procedure described by Mohanraj et al. 2010^[6], was used for determining the antifungal activity. Different dilutions (2, 3, 4 & 5 mg/ml) of the various extracts of *A. siliquosa* was made and 1 μ l of each dilution was added to each well. One of the wells in which no extract was added was used as the control. The plates were incubated at 37°C for 72 hrs. Nystatin dihydrate was used as positive control. A red colour indicated no growth and yellow colour was considered positive. Optical density was measured and percentage of fungal inhibition was calculated by comparing OD of the treated wells with the control well. The experiments were conducted in triplicates.

III. RESULTS

The antifungal activity, shown by the different extracts of *A.siliquosa* is presented in Table 1 and 2. Out of the different extracts analyzed, methanol extracts showed the best activity against *C. albicans*, *A. niger* and *E. floccosum*.

Table 1 Effect of Various Extracts of *Apama Siliquosa* on *In Vitro* Growth of Selected Fungal Pathogens (% Inhibition)

Fungi	Nystatin ^a 50ppm	Methanol extracts (ppm)				Ethylacetate (ppm)				Chloroform extracts(ppm)			
		2000	3000	4000	5000	2000	3000	4000	5000	2000	3000	4000	5000
<i>Candida albicans</i>	100 ^b	74.5	100 ^b	100	100	51.5	65.2	80	100 ^b	53.4	68.5	85.7	100 ^b
<i>Aspergillus niger</i>	100 ^b	63.4	80.8	91.2	100 ^b	63.5	75.3	82.6	100 ^b	65.2	72.7	85.8	100 ^b
<i>Trichophyton mentagrophytes</i>	100 ^b	33.5	52.0	60.5	80	30.3	55.3	68.6	76.2	55.1	61.2	81.3	94.7
<i>Epidermophyton floccosum</i>	100 ^b	72.8	86.7	100 ^b	100	63.2	78.1	86.3	100 ^b	65.4	81.7	100 ^b	100
<i>Microsporum gypseum</i>	100 ^b	53.5	72.3	83.7	92.1	42.1	55.2	73.6	78.4	50.3	62.6	68.8	95.3

The values are the average of three determinations

^a Positive control

^b Fungicidal effect

Table 2 Effect of Hot Water Extracts Of *Apama Siliquosa* on *In Vitro* Growth of Selected Fungal Pathogens (% Inhibition)

Fungi	Nystatin ^a 50ppm	Hot water extracts (ppm)			
		2000	3000	4000	5000
<i>Candida albicans</i>	100 ^b	62.1	75.0	100 ^b	100
<i>Aspergillus niger</i>	100 ^b	42.4	68.1	82.8	100 ^b
<i>Trichophyton mentagrophytes</i>	100 ^b	43.7	65.0	73.8	86.2
<i>Epidermophyton floccosum</i>	100 ^b	61.8	77.1	82.4	100 ^b
<i>Microsporum gypseum</i>	100 ^b	46.4	58.1	75.6	88.1

The values are the average of three determinations

^a Positive control

^b Fungicidal effect

IV. DISCUSSION

Screening of bioactive agents from plant is one of the most intensive areas of natural product research today. The presence of antibacterial substances in the higher plants is well established^[7]. Thus, they can be used in the treatment of infectious diseases caused by microbes. Methanol extract of the roots of *A. siliquosa* showed the best antifungal activity against most organisms. The presence of glycoside alkaloids, flavonoids and phenols from the extracts of *A. siliquosa*^[8] could be attributed to its potent antimicrobial activities. The reputation of *A.siliquosa* as a remedy for stomach ache, dysentery and other microbial diseases was supported by the antifungal screening tests.

Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but we found in this study the plant extracts by methanol provided more consistent antifungal activity compared to those extracted by water.

V. CONCLUSION

A.siliquosa extracts showed antifungal activity against all the tested pathogenic fungi and the activity was dose dependent. Though the medicinal properties of *A. siliquosa* have been well documented, studies on the bioactivities of the plant are limited. The present study is a pioneer work in this kind and gives credential to the use of *A.siliquosa* in tribal medicine.

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