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Evaluation of the Antimycotic Activity of Aqueous and Ethanolic Extracts of *Aesculus hippocastanum* – *An In Vitro* Study

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Abstract

The aim of the present study was to assess the antimycotic activity of aqueous and ethanolic extracts of *Aesculus hippocastanum* (horse chestnut). Horse chestnut herbal remedies are utilized in traditional folk medicine. Many parts of the horse chestnut tree, including the seeds, leaves and bark have been used medicinally. The Aqueous and Ethanolic extracts of horse chestnut were used to find out the antimycotic activity against *Candida albicans, Aspergillus niger, Aspergillus fumigates, Mucor sps and Penicillium morneffi*. Agar well diffusion technique was followed for screening the antimycotic activity. The prepared wells were loaded with 50µl of aqueous and ethanolic extracts at different concentrations .The extracts at different concentrations showed varying degree of antimycotic activity against the fungi tested, compared to the standard.

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Introduction

Antimicrobial activities of various herbs and spices in plant leaves, flowers, stems, roots, or fruits have been reported by many workers. [1] A wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, quinones and flavonoids are endowed with antimicrobial properties.[2, 3] The plant under present

study, *Aesculus hippocastanum* also has some antimicrobial property.

Aesculus hippocastanum (family Hippocastanacae) is commonly known as Horse chestnut, which is native to Western Asia. Horse chest nut is also known as Spanish chestnut, buckeye, seven leaves tree, is a deciduous tree which grows up to a height of 35 meters with a large regular crown, five to seven digitate leaves and erect racemes of flowers with a yellow or reddish spot at the base of the white petals. The fruit is a spiny capsule containing up to three shiny, reddish brown seeds with a light-colored hilum. [4,5] It is indigenous to the mountains of Greece, Bulgaria, the Caucasus, northern Iran and the Himalayas[5]

The seeds have been used as an analgesic, antipyretic, narcotic, tonic, and vasoconstrictor. They have been used to treat backache, sunburn, neuralgia, rheumatism, whooping cough and hemorrhoids. [6, 7, 8] The bark has been used as a tonic, narcotic, antipyretic

and induce sneezing. The flowers have been used as an anodyne, astringent, tonic and

vulnerary[7]. The extracts of Horse chestnut have been traditionally employed both in the West and East for the treatment of peripheral vascular disorders including haemorrhoids, varicose veins, leg ulcers and bruises[9] .It is used in the treatment for chronic venous insufficiency and peripheral edema[10]. It has antilipemic, expectorant, diuretic properties and antimicrobial activity. It is also used for the prevention of gastric ulcers, reduction of cerebral edema, reduction of cellulite, as adrenal stimulant, hypoglycemic agent, antithrombotic, anti-inflammatory, and also for reduction of hematomas and inflammation from trauma or surgery.

Active Chemical Constituents of horse chest nut are coumarin derivatives like aesculin, fraxin,

scopolin; flavonoids like quercetin, kaempferol,

astragalin, isoquercetrin, rutin, leucocyanidine and essential oils like oleic acid, linoleic acid.Other constituents include amino acids (adenosine, adenine, guanine), allantoin, argyrin, carotin, choline, citric acid, epicatechin, leucodelphinidin, phyosterol, resin, scopoletin, tannin, and uric acid. [6,11,12]The principal extract and medicinal constituent of horse chestnut seed is aescin, a mixture of triterpenoid saponin glycosides. Its components include protoaescigenin, barringtogenol C, allantoin, sterols, leucocyanidin, leucodelphinidin, tannins, and alkanes.[5, 6, 12, 13] The present study was conducted to assess the antimycotic activity of aqueous and ethanolic extracts of *Aesculus hippocastanum* (horse chestnut) against different fungal strains.

MATERIALS AND METHODS

Plant materials:

Aqueous and ethanolic extracts of *Aesculus hippocastanum* was obtained from Green Chem Herbal Extract & Formulations, Bangalore.

Test microorganisms

Fungal strains used were *Candida albicans, Aspergillus niger, Aspergillus fumigates, Mucor sps and Penicillium morneffi.* The organisms were obtained from Department of Microbiology, Saveetha Medical College & Hospitals, and maintained in SDA slope at 4°C.

Methodology

The extracts were prepared in the following concentrations in sterile water.2.5 mg/ml, 5 mg/ml and 10 mg/ml, so that 50µl of extract of different concentrations delivers 125µg, 250µg and 500 µg respectively.

Anti mycotic Assay - Agar well Diffusion Technique:

The extract at different concentrations was screened for their antimycotic (antifungal) activity against the selected fungal strains by Agar well diffusion method.[14,15] The fungal cultures were grown on Sabourauds destrose agar [Hi media M063]. The fungal growth from seven day old culture was washed, suspended in normal saline and then filtered through glass wool aseptically. The colony forming

units (CFU/ml) of suspension of the fungus was determined and test inoculum was adjusted to 0.5 Mc Farland's standard[16,17] and used for antifungal assay.100µl of the test inoculum were applied on the surface of the Sabourauds destrose agar plate and spread using sterile glass spreader. Wells were cut on the agar plates using sterile cork borer for different concentration of the extracts. 50µl of extract of different concentrations were loaded in to the wells and incubated for 48 h at 28°C. As a positive control, fluconazole (10 mcg /disc) and amphotericin B (100 units /disc) were used. Zone of inhibition in mm were determined after 48 h. The test was performed in triplicate to minimize test error.

RESULT AND DISCUSSION

The antimycotic activity of the extracts (Ethanolic and Aqueous) at different concentrations was screened by agar well diffusion technique and the zone of inhibition was measured in mm diameter. The results are given in the Table 1, Figure 1 and Figure 2

Table 1. Antimycotic activity of Aqueous and Ethanolic extracts of *A. hippocastanum*

Extract	Concentration [µg]	Zone of inhibition [in mm diameter]				
		B1	B2	B3	B4	B5
Ethanolic	125	14	12	14	10	11
	250	18	17	16	13	14
	500	22	19	20	14	22
Aqueous	125	9	7	9	7	10
	250	12	14	12	10	14
	500	16	21	15	12	16
Fluconazole	10mcg/disc	21	21	20	19	20
Amphotericin B	100 units/disc	20	20	19	18	18

B1-*Candida albicans,* B2-*Aspergillus niger,* B3- *Aspergillus fumigates,*B4- *Mucor sps*

B5 - *Penicillium morneffi*

Figure 1 : Graph showing the Antimycotic activity of Ethanolic extract of *A. hippocastanum*

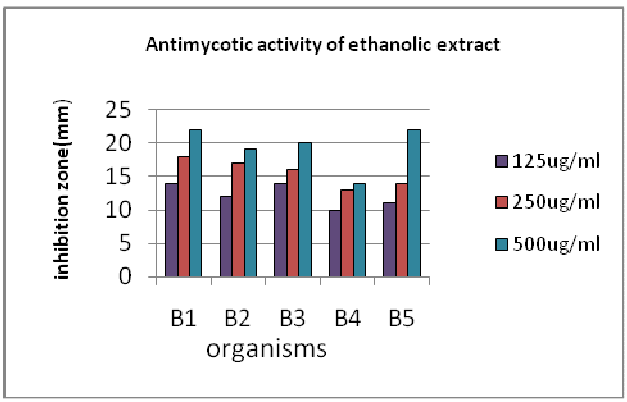
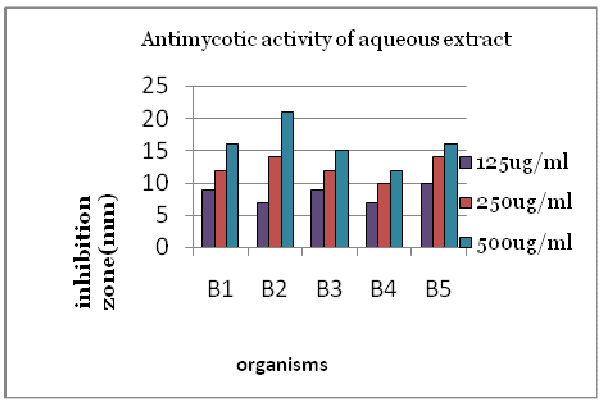


Figure 2 : Graph showing the Antimycotic activity of Aqueous extract of *A.hippocastanum*



CONCLUSION

The present study conclude the efficacy of *Aesculus hippocastanum* as a potent herb with antimycotic activity and may be used in treating diseases caused by the test organisms. Further studies are required to isolate, identify and elucidate the structure of the bioactive compound particularly responsible for the antimycotic activity of this medicinal plant.

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