

## IN VITRO EVALUATION OF ANTIBACTERIAL ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *AESCULUS HIPPOCASTANUM* ON ORAL MICROBES

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### ABSTRACT

The aim of the present study was to assess the antibacterial activity of aqueous and ethanolic extracts of *Aesculus hippocastanum* (horse chestnut) on oral microbes. Horse chestnut herbal remedies are utilized in traditional folk medicine. Many of the 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 antibacterial activity against oral microbes like *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, *Streptococcus sanguis*, and *Lactobacillus acidophilus*. Agar well diffusion technique was followed for screening the antibacterial activity. The prepared wells were loaded with 50µl of aqueous and ethanolic extracts at different concentrations. The extract at different concentrations showed varying degree of antibacterial activity against the micro organisms tested compared to standard.

**Keywords:** Horse chest nut, Extracts, Agar well diffusion, Antibacterial activity, Oral microbes

### INTRODUCTION

Plants have been traditionally proved to be a rich source of novel drug compounds, as the herbal mixtures have made large contributions to human health and well-being<sup>1</sup>. A wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, quinones and flavonoids are endowed with antimicrobial properties.<sup>2,3</sup>. The plant under present study, *Aesculus hippocastanum* also has antimicrobial property.

*Aesculus hippocastanum* (family Hippocastanaceae) is commonly known as Horse chestnut which is native to Western Asia<sup>9</sup>. Horse chest nut is also known as Spanish chestnut, buckeye, seven leaves tree, is a deciduous tree up to 35 meters high 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<sup>4,5</sup>. Horse chestnut is widely cultivated as an ornamental tree, especially in northern Europe and North America. It is indigenous to the mountains of Greece, Bulgaria, the Caucasus, northern Iran and the Himalayas<sup>5</sup>

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,<sup>6, 7, 8</sup> The bark has been used as a tonic, narcotic, antipyretic and to induce sneezing. The flowers have been used as an anodyne, astringent, tonic and vulnerary<sup>7</sup>. 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<sup>9</sup>. It is used in the treatment for chronic venous insufficiency and peripheral edema<sup>10</sup>. 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, argyran, carotin, choline, citric acid, epicatechin, leucodelphinidin, phyosterol, resin, scopoletin, tannin, and uric acid<sup>6,11,12</sup>

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<sup>5, 6, 12, 13</sup>

It can be fractionated into beta-aescin, an easily crystallizable mixture, and alpha-aescin, which is watersoluble<sup>11</sup>. Aescin decreases the transcapillary filtration of water and proteins.

It has been used to treat a wide variety of inflammatory and edematous conditions, to reduce swelling associated with bruises, fractures, brain trauma, post-operative and post-traumatic soft tissue swelling, and acute thrombophlebitis<sup>8,14</sup>. Aescin reduces lysosomal enzyme activity by stabilizing lysosomal membranes and limiting enzyme release<sup>15</sup>. Aescin also improves venous tone by enhancing the constricting effect of noradrenaline.

### MATERIALS AND METHODS

#### Plant Material

The ethanolic and aqueous extracts of *Aesculus hippocastanum* (Horse chestnut) were obtained from Green Chem Herbal Extract & Formulations, Bangalore.

#### Test Microorganisms

Bacterial strains used were *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, *Streptococcus sanguis* and *Lactobacillus acidophilus*. The organisms were obtained from Department of Microbiology, Saveetha Dental College and maintained in nutrient agar slope at 4°C.

#### Methodology

The extracts were prepared in the following concentrations in sterile water. 2mg /ml, 3mg /ml and 4mg /ml, so that 50µl of extract of different concentrations delivers 100µg, 150µg and 200 µg respectively.

#### Assay for the Antibacterial Activity Using Agar Well Diffusion Method

The screening of antibacterial activity of plant extracts was carried out using the agar well diffusion method. The bacterial strains were inoculated into tubes of Brain heart infusion agar and incubated at 37°C overnight. Each of the cultures were then adjusted to 0.5 McFarland turbidity standard.<sup>17-20</sup> Lawn culture of the test organisms were made on the Brain heart infusion agar [BHI-Hi media M211] plates using sterile cotton swab and the plates were dried for 15 minutes. A sterile cork borer was then used to make wells (6mm diameter) for different concentrations of the extracts on each of the plates containing cultures of the different bacterial

strains. 50µl of the varying concentrations (100,150, 200µg) of the extracts were introduced into the wells with the help of micropipette. The culture plates were allowed to stand on the working bench for 30 min for pre-diffusion and were then incubated in upright position at 37°C for 24 h. After 24 hrs, antibacterial

activity was determined by measurement of diameter of zones of inhibition (mm). Standard antibiotic discs of Amoxicillin (30mcg/disc) and Penicillin G (30mcg/disc) were used as positive control. All the tests were done in triplicate to minimize the test error.

**Table 1: Antibacterial Activity OF Aqueous and Ethanolic Extracts of *Aesculus Hippocastanum***

Extract	Concentration [µg]	Zone of inhibition [in mm diameter]				
		B1	B2	B3	B4	B5
Ethanolic	100	10	12	8	14	12
	150	13	15	10	17	14
	200	20	19	12	20	18
Aqueous	100	8	10	7	11	11
	150	10	12	9	14	13
	200	16	16	10	18	17
Penicillin G	30mcg/disc	24	21	22	23	20
Amoxycillin	30mcg/disc	25	23	20	21	18

B1- *Streptococcus mutans*, B2- *Streptococcus salivarius* , B3-*Streptococcus mitis* , B4- *Streptococcus sanguis*, B5- *Lactobacillus acidophilus*

## RESULT AND DISCUSSION

The antibacterial 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.

Both the extracts at different concentration showed anti bacterial activity against the bacterial strains tested. The ethanolic extract was more effective against *Streptococcus mutans* and *Streptococcus sanguis* with a zone of inhibition of 20 mm diameter (at conc. 200 µg.) and was least effective against *Streptococcus mitis* with zone of inhibition of 12mm (at conc. 200 µg.) and among the other bacterial species studied *Streptococcus salivarius* showed a zone of inhibition of 19mm diameter (at conc. 200 µg.) and *Lactobacillus acidophilus* showed inhibition zone of 18mm diameter (at conc. 200 µg.).

The mouth harbors a diverse, abundant and complex microbial community. This highly diverse microflora inhabits the various surfaces of the normal mouth. Bacteria accumulate on both the hard and soft oral tissues in biofilms. Bacterial adhesion is particularly important for oral bacteria. Oral microorganisms have major role in the dental diseases like dental caries and periodontal disease. Oral bacteria include streptococci, lactobacilli, staphylococci, corynebacteria, and various anaerobes in particular bacteroides. The oral cavity of the new-born baby does not contain bacteria but rapidly becomes colonized with bacteria such as *Streptococcus salivarius*. With the appearance of the teeth during the first year colonization by *Streptococcus mutans* and *Streptococcus sanguis* occurs as these organisms colonise the dental surface and gingiva. Other strains of streptococci adhere strongly to the gums and cheeks but not to the teeth. The gingival crevice area (supporting structures of the teeth) provides a habitat for a variety of anaerobic species. Bacteroides and spirochetes colonize the mouth around puberty.<sup>22</sup>

The teeth are the only non shedding surfaces in the body, and bacterial levels can reach more than 10<sup>11</sup> microorganisms per mg of dental plaque. Human endodontal and periodontal infections are associated with complex microfloras in which approximately 200 species (in apical periodontitis) and more than 500 species (in marginal periodontitis) have been encountered. The anatomic closeness of these microfloras to the bloodstream can facilitate bacteremia and systemic spread of bacterial products, components, and immunocomplexes<sup>23</sup>. So control of oral microbes is very essential for healthy life.

There has been an increase in the use of herbal medicines over the last 15-20 years. There is a public belief that these medicines are safe because they are made from natural sources. Numerous reports have shown the use of traditional plants and natural products for the treatment of oral diseases. The present study was to evaluate the antibacterial activity of ethanolic and aqueous extracts of *Aesculus hippocastanum* on selected oral microorganisms.

## CONCLUSION

The present study shows the antibacterial activity of *Aesculus hippocastanum* (horse chestnut) against the oral microorganisms which have a major role in the dental diseases like dental caries and periodontal disease. So, use of these extracts in appropriate concentration in a better pharmaceutical dosage form after further toxicity studies may be promising to control not only the oral diseases but also the prevention of systemic disease for which some these organisms are responsible.

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