

## Antibacterial and antifungal effects of the leaf, seed, seed coat and fruit capsule of *Aesculus hippocastanum* (Sapindaceae) extracts

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**Abstract:** The present study describes the antimicrobial activity of the leaf, seed, seed coat and fruit capsule of horse-chestnut (*Aesculus hippocastanum*, L.) extracts from the Ordu Province of Turkey. These extracts were first prepared with ethanol solvent. The antimicrobial activity of the extracts was then assessed using the disc diffusion method against 7 bacteria and 1 yeast. The antimicrobial test results revealed that each the crude extracts of *A. hippocastanum* exhibited very weakly possess antibacterial activity in the case of both Gram-positive and Gram-negative bacteria. The most sensitive microorganism to *A. hippocastanum* samples were *Proteus vulgaris* and *Listeria monocytogenes*. The results presented in this paper suggest the possibility that *A. hippocastanum* possesses a new antimicrobial compound that has a weakly effect against some Gram-negative and Gram-positive bacteria, but did not against fungi.

**Keywords:** Sensitive microorganisms, *Proteus vulgaris*, *Listeria monocytogenes*.

### Introduction

Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases. Approximately 60-80% of the world's population still relies on traditional medicines for the treatment of common illnesses (WHO, 2002-2005; Zhang, 2004). Natural products have served in the past as abundant source of compounds proven to be useful in the treatment of human disease. A novel source of natural plant products field collections of which have yield extracts with antineoplastic, antimicrobial, antifungal and antiviral activities (Lau et al., 1993).

Since plants have a variety of chemical compounds in their leaves, roots and flowers, they have been used in the treatment of various human diseases for thousands of years in all over the world. Similarly, a lot of plants have been used by local people in Turkey for the purpose of the treatment of several diseases, including microbial infections for emetic and strengthening effects, and for increasing urine and decreasing tension (Baytop, 1984; Peirce, 1999). *Aesculus hippocastanum* L. commonly known as horse chestnut is not the same as the edible sweet chestnut, *Castanea sativa* Mill. Raw horse chestnuts

are having poisonous effects, but after special preparation to remove the toxins (WHO, 2002-2005). An ancient superstition of carrying a horse chestnut seed around in one's pocket to prevent or cure arthritis still exists in some countries (Peirce, 1999; Robbers and Tyler, 1999). 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 haemorrhoids (Peirce, 1999; Duke, 1985). 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 (Peirce, 1999).

The first compound with antimicrobial activity was found in the 1930s (Goodman et al., 1991). Since that period the development and use of these substances has increased, especially with the appearance of resistant strains. Plants which have been used as medicines over hundreds of years constitute an obvious choice for study. It is interesting to determine whether their traditional uses are supported by actual pharmacological effects or merely based on folklore (Zinhener and Mear, 1972).

In the present study, we have investigated the antimicrobial effect of ethanol extracts leaf, seed, seed

coat, fruit capsule of *A. hippocastanum* L. (Sapindaceae) on Gram-positive bacteria, Gram-negative bacteria and fungi (*Aspergillus niger*). Antimicrobial effect of this plant appears have not been studied.

### Materials and Methods

**Plant materials:** The leaf, seed, seed coat, fruit capsule of horse-chestnut (*A. hippocastanum*.) was collected in during in summer 2007 from the Ordu Province of Turkey. The identification of this specimen was carried out using Flora of Turkey (Davis, 1966-1988).

**Preparation of extract:** The leaf, seed, seed coat, fruit capsule of horse-chestnut of the plants were all samples were air-dried and powdered. The extract of the plants was prepared according to the methods described by Holopainen et al. (1988) with slight modification. Dried the leaf, seed, seed coat, fruit capsule of horse-chestnut of the plants were extracted with ethanol (50 g 1/5 solvent) at room temperature. The extracts were kept at 4°C for 5 days, and they were filtered through 45 µm membrane filter, and then the solution was dried with an evaporator. The crude extracts were stored at -20°C until used.

**Test strains and culture media:** Strains of bacteria and fungi were obtained from ATCC (American Type Culture Collection, Rockville, Maryland). Antimicrobial activities of four extracts of *A. hippocastanum* samples were assayed against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Staphylococcus epidermidis* ATCC 12228, *Bacillus cereus* ATCC10876, *Klebsiella pneumoniae* ATCC 5041, *Proteus vulgaris* B123, *Listeria monocytogene* NCTCS 11994 and *Aspergillus niger* ATCC 16404. The species of bacteria were grown in Mueller Hinton Agar (Merck) and Mueller Hinton Broth (Merck), and *A. niger* was grown in Sabouraud Dextrose Broth (Difco) and Sabouraud Dextrose Agar (Oxoid). The concentration of bacterial suspensions was adjusted to 10<sup>8</sup> cells/mL, and that of fungal suspensions to 10<sup>7</sup> cells/ml.

**Antifungal and Antibacterial assay:** Antibacterial and antifungal activities were measured using methods of diffusion disc plates on agar (Ronald, 1990). In order to test antibacterial and antifungal activity, four extracts of *A. hippocastanum* samples were dissolved in ethanol 70%. For bacterial Mueller Hinton Agar medium (Merck) (20 ml and Sabouraud Dextrose Agar Oxoid 20 ml) for fungus were poured into each 15 cm Petri dish. All bacterial strains were grown in Mueller Hinton Broth

medium (Merck) for 24 h, at 37°C and, *A. niger* was grown in Sabouraud Dextrose Broth (Difco) at 27°C for 48 h. Growth was adjusted to OD (600 nm) of 0.1 by dilution with Mueller Hinton Broth medium (Merck) for bacteria and for fungi Sabouraud Dextrose Broth (Difco). Suspension (100 µl) with approximately 10<sup>8</sup> bacteria and fungi per millilitre was placed in Petri dishes, over agar and dispersed. Then, sterile paper discs (Oxoid, CT09988, 6 mm diameter) were placed on agar to load 15 µl of each plant samples (1 mg/ml). One hundred units of nystatin, for bacteria Ampicillin and Cephazolin obtained from a local pharmacy were used as a positive control and alcohol as a negative.

**Statistical analysis:** The data were analysed using SPSS for Windows (v.13.0). The differences between the means of the inhibition zones were tested with one-way variance analysis followed by Tukey's HSD test. The results were evaluated in the confidence limit of 0.05.

### Results and Discussion

In the present study, the antimicrobial activity of the leaf, seed, seed coat, fruit capsule of ethanol extracts of *A. hippocastanum* from the Ordu Province of Turkey were investigated. The antibacterial and antifungal activity on four different *A. hippocastanum* extracts were initially evaluated by the disk diffusion method using eight strains of (Gram-positive, Gram-negative) bacteria and one fungus strain (*A. niger*). Although *A. hippocastanum* extracts exhibited very weaken antibacterial activity, they did not exhibit antifungal activity. The results obtained in the disk diffusion assay regarding the growth inhibition zones of the tested microbes are shown in Table 1. Generally, 1 some ethanol extracts of *A. hippocastanum* sample did not exhibit inhibitory action on the *Bacillus cereus*, *Staphylococcus epidermidis*, *Proteus vulgaris* and *Streptococcus pneumoniae* but the samples showed weak inhibitory effect on the growth of *Listeria monoocytognes*, *Klebsiella pneumoniae* and *Staphylococcus aureus* (12-8 mm/15 µl inhibition zone). On the other hand, the leaf, seed, seed coat, fruit capsule of ethanol extracts of *A. hippocastanum* did not showed antifungal activity against *A. niger* (-mm/15 µl inhibition zone). However, 2 ethanol of *A. hippocastanum* sample weakly exhibit inhibitory action on the *P. vulgaris*. But did not ethanol extract exhibit inhibitory action on the other test organisms. The same time, 3 and 4 ethanol of *A. hippocastanum* sample

**Table 1.** Results of antimicrobial screening the leaf, seed, seed coat and fruit capsule extracts of *A. hippocastanum* determined by the agar diffusion method (inhibition zone in mm).

Samples	Microorganisms Inhibition Zone (mm)							
	<i>A. niger</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. epidermidis</i>	<i>P. vulgaris</i>	<i>K. pneumonia</i>	<i>L. monocytogene</i>	<i>B. cereus</i>
1 Fruit capsule	7.0±0.2 <sup>c</sup>	8.0±0.5 <sup>b</sup>	7.0±0.2 <sup>c</sup>	6.0±0.2 <sup>b</sup>	7.0±0.3 <sup>b</sup>	8.0±0.5 <sup>b</sup>	12.0±0.5 <sup>de</sup>	6.0±0.2 <sup>b</sup>
2 Seed coat	6.0±0.2 <sup>b</sup>	8.0±0.5 <sup>b</sup>	6.0±0.2 <sup>b</sup>	7.0±0.2 <sup>c</sup>	12.0±0.5 <sup>de</sup>	6.0±0.2 <sup>b</sup>	6.0±0.2 <sup>b</sup>	7.0±0.5 <sup>b</sup>
3 Seed	7.0±0.2 <sup>c</sup>	7.0±0.2 <sup>c</sup>	9.0±0.5 <sup>cd</sup>	7.0±0.2 <sup>c</sup>	7.0±0.2 <sup>c</sup>	7.0±0.3 <sup>b</sup>	8.0±0.5 <sup>b</sup>	7.0±0.2 <sup>c</sup>
4 Leaf	7.0±0.2 <sup>c</sup>	8.0±0.5 <sup>b</sup>	9.0±0.3 <sup>c</sup>	7.0±0.3 <sup>b</sup>	7.0±0.3 <sup>b</sup>	7.0±0.2 <sup>c</sup>	9.0±0.3 <sup>c</sup>	8.0±0.5 <sup>b</sup>
Ampicillin	NT	11.0±0.5 <sup>cd</sup>	15.3±0.3 <sup>dd</sup>	35.3±0.3 <sup>h</sup>	28.0±0.3 <sup>f</sup>	13.0±0.5 <sup>f</sup>	24.7±0.3 <sup>e</sup>	25.0±0.3 <sup>e</sup>
Cephazolin	NT	8.0±0.5 <sup>b</sup>	15.3±0.3 <sup>c</sup>	35.3±0.3 <sup>h</sup>	7.0±0.2 <sup>c</sup>	10.0±0.4 <sup>b</sup>	30.00±0.3 <sup>g</sup>	21.7±0.3 <sup>d</sup>
Nystatin	15.3±0.3 <sup>d</sup>	NT	NT	NT	NT	NT	NT	NT
70% ethanol	-	-	-	-	-	-	-	-

The differences between the means in the same column by the same letter are not statistically significant  $P>0.05$ . -: no inhibition, NT: Not tested: *S. aureus* ATCC25923, *K. pneumonia* ATCC5041, *E. coli* ATCC25922, *P. vulgaris* B123, *S. epidermidis* ATCC12228, *B. cereus* ATCC10876, *L. monocytogene* NCTC11994 and *A. niger* ATCC16404.

weakly exhibit inhibitory action on the test organisms (the Table 1).

The leaf, seed, seed coat and fruit capsule did not show highly activity in alcoholic extract. However, negative results do not indicate the absence of bioactive constituents, nor that the plant is inactive. Active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed (Taylor et al., 2001; Parekh and Chanda, 2008). Maximum antibacterial activity amongst alcoholic extract was shown by 1, followed by 2 for *P. vulgaris* and *L. monocytogene* (Table 1). These extracts may be contain a little of flavonoids because flavonoids are possess antibacterial activity (Harborne and Baxter, 1993). These antibacterial and antifungal studies are the first reported for this plant. Significant antibacterial and antifungal activities did not found by the experiment. But this plant extract was used in experiment by many of scientist. They found that extracts of the seeds of *A. hippocastanum* (horse-chestnut) are a well-established treatment for conditions such as varicose veins, haemorrhoids, phlebitis (inflammation of the veins), diarrhoea, fever, enlargement of the prostate gland (Newall et al., 1996), rheumatism, neuralgia and rectal complaints (Bisset, 1994). Such treatments are usually administered orally, at a dose of 0.5-1.2 ml of liquid seed extract per day (Wilkinson and Brown, 1999). The seeds

have been used as an analgesic, antipyretic, narcotic, tonic, and vasoconstrictor. They have been used to treat backache, sunburn, neuralgia, rheumatism, vulnerary (Peirce, 1999), whooping cough and haemorrhoids (Peirce, 1999; Duke, 1985). 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 the bark, buds, leaves, seeds, and the immature fruit pericarp of *A. hippocastanum* contain saponins, a number of flavonoids and other constituents (Wilkinson and Brown, 1999). 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* by Anitha Roy et al. (2011). Most of these compounds are found in the seeds (not including the integument), but have also been found in the fruit pericarp, bark, leaves and buds. Results concerning quercitrin are contradictory, with different authors reporting that this compound is present either in the leaves or in the seeds of *A. hippocastanum* (Peirce, 1999; Anitha Roy et al., 2011).

### Conclusion

Plants, herbs, and ethnobotanicals have been used since the early days of humankind and are still used throughout the world for health promotion and treatment of disease.

Horse chestnut extracts are widely used in pharmacy and cosmetic industries plants and natural sources form the basis of today's modern medicine and contribute largely to the commercial drug preparations manufactured today. Extracts from *A. hippocastanum*, and in particular, those based on horse chestnut seeds, contain saponins, known collectively as aescin, which have a gentle soapy feel, and are potent anti-inflammatory compounds. The results indicated that each the crude extracts of the leaf, seed, seed coat and fruit capsule extracts of *A. hippocastanum* (horse-chestnut) exhibited more or less pronounced antibacterial and antifungal potencies in the case of both gram-positive and gram-negative bacteria and fungus.

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