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ANTIOXIDANT AND ANTI-BACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF AESCULUS INDICA

Aamna Khokhar[1], Naseer Ahmed[2], Iram Kamal[1], Rizwan Faisal[3], Afeefah Sattar[1]

1. Department of Pharmacology, Bahauddin Zakariya University, Multan, Pakistan

2. Department of Pharmacology, Rehman College of Dentistry, Peshawar, Pakistan

3. Department of Pharmacology, Rehman Medical College, Peshawar, Pakistan

Corresponding Author: naseer.ahmad@rmi.edu.pk

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ABSTRACT

BACKGROUND: Oxidative stress and bacterial infections have been identified as the root cause of the development and progression of several diseases. Despite many medicinal plants being reported to have antioxidant and antibacterial potentials, only a few are investigated. Medicinal plants exerting these properties can be used alone or in combination with other drugs for the treatment of various disorders.

OBJECTIVE: To determine the antioxidant and anti-bacterial activity of ethanolic extract of Aesculus Indica.

METHODOLOGY: This experimental, lab-based study was carried out at the Pharmacy department of Bahauddin Zakariya University Multan, from September 2022 to March 2023. The ethanolic extract of Aesculus indica was evaluated for its antioxidant and antibacterial activity. The stable 1,1-diphenyl -2-picrylhydrazyl radical was used to evaluate the antioxidant activity. Quercetin was used as standard antioxidant. Antibacterial activity of Aesculus indica was evaluated by paper disc diffusion method against the bacterial cultures of Staphylococcus epidermidis, and Bacillus atrophaeus at the dose of 100mg/ml and 200mg/ml. Ciprofloxacin (40µg/ml) was used as a standard antibacterial drug for Staphylococcus epidermidis.

RESULTS: The mean of Aesculus Indica extract for 1,1-diphenyl -2-picrylhydrazyl radical scavenging activity was 83.76±0.76 while that of standard drug quercetin was 93.21±0.97. Statistically significant difference was found between the two. Ethanolic extract of Aesculus Indica showed significant ($p<0.05$) antibacterial activity in comparison to ciprofloxacin against Staphylococcus epidermidis and Bacillus atrophaeus.

CONCLUSION: Ethanolic extract of the Aesculus Indica showed significant antioxidant and anti-bacterial

KEYWORDS: EAesculus indica, Antioxidant, Antibacterial, Quercetin, Ciprofloxacin.

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INTRODUCTION

Plants being the core and crucial component of complementary and alternative medicine acquiring the competency of forming secondary metabolites including proteins, flavonoids, alkaloids, steroids, and phenolic compounds that are useful in regaining health and handling many ailments.[1-4] These days, medicinal plants are gaining popularity again for their therapeutic values as they are believed to have less adverse effects.[5-6] The species of *Aesculus indica (A. indica)* under the family *Hippocastanacea,* subfamily of *Sapindaceae,* is commonly found as herbaceous, soft perennial plant in Northwestern Himalayas. Commonly it is known as Bankhor. It is well established that it offers numerous health benefits to humans. The seeds are employed as astringent, food, while the oil is employed in the remedy of skin diseases and rheumatism.[7] *A. indica* is native to the woodland habitat preferably called Indian horse chestnut or Himalayan chestnut. In different parts it is known by different names like hanudon in Kashmir, goon in H.P, khajushing in Bhutan, pangro in Nepal, and jawaz in Pakistan. This plant falls under the Genus *Aesculus* which has species in the regions of America, Europe, and Asia. Folk medicinal uses of *A. indica* include indigestion, stomachache, arthritis, venereal diseases, chest problems, skin ulcers, hemorrhoids, and jaundice.[8]

A. Indica is distributed mainly in the colder areas of the world. Due to the existence of medicinal attributes, it is frequently used in folk medicines. It is very helpful in rheumatism, Gastrointestinal disorders, reproductive diseases, diabetes, cancers, hemorrhoids, varicose veins and in ulcers to avoid formation of thrombus.[9] It frequently proves helpful in the treatment of migraine, blood effusions as well as frost bite illnesses.[10] Patients with acute and intermittent fever are treated with the bark of the tree in France and Germany.[11] Additionally, pharmacological research by Firdoos et al has revealed that *A.indica* possesses a variety of effects including cytotoxic, anti-bacterial, immune-modulatory, anti-viral, neurodepressive, spasmolytic, and anti-inflammatory. Its ethnomedicinal use was supported by the evaluation of *A.indica*'s anti-nociceptive activity, which was mediated by two separate peripheral and central analgesic pathways, thus supporting its major use in analgesic activities.[12] This may be the reason that it is used against several infections.[13]

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A. Indica also inherits antioxidants properties. It contains potent antioxidant bioactive compounds like mandelic acid, quercetin and aescin which hold a promising therapeutic value in handling oxidative stress environments.[14] Limited work is done on its antioxidant and antibacterial properties. Therefore, the present study was conducted to investigate antioxidant properties of *A. indica*. The results of the study will provide a good insight to the treatment of various reproductive, hepatobiliary, gastrointestinal, and central nervous system disorders.

METHODOLOGY:

This experimental, lab-based study was carried out at the Pharmacy department of Bahauddin Zakariya University Multan, from September 2022 to March 2023. Entire plant of *A. indica* (5kg) was collected from Murree and its identity was confirmed by Botany Department, University of Haripur.

Preparation of plant ethanolic extract:

Plant substance rendered free from soil and polluted material, dried under shade and was then coarsely powdered. Then was soaked in ethanol (70%) for 7 days with intermittent shaking. It was then passed through a muslin stuff and its liquid portion through a sift piece. Filtrate was evaporated under reduce pressure (760mmHg), then transferred to petri dish and positioned at room temperature to evaporate the left-over filtrate. The extraction procedure was repeated two times to obtain greatest yield. The ethanolic extract was transferred to a glass bottle and stored in refrigerator (-4 ^{0}C) until used (Figure 1).

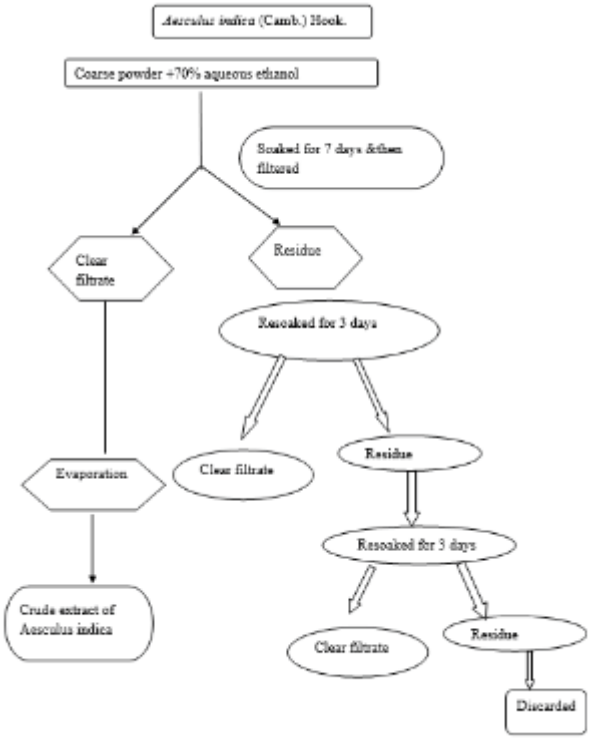

Figure 1: Preparation of ethanolic extract of *A.indica*

Antioxidant activity by DPPH Method:

The stable 1,1-diphenyl -2-picrylhydrazyl radical (DPPH) was used to establish antioxidant activity. Different concentrations of compound in respective solvent were added at an equal volume (10µl) to 90µl of 100µM ethanolic DPPH in a total volume of 100µl in 96 – well plates.[15] The contents were mixed and incubated at 37 $^{\circ}$C for 30 min. The absorbance was measured at 517 nm using Synergy HT Bio Tek® USA. Micro plate reader. Quercetin and L – ascorbic acid was used as standard antioxidants. The experiments were carried out at triplicate. The reduced absorptivity indicates enhanced free radical neutralizing capability which was established by the following expression:

% free radical neutralization = [100- (Abs of test compound / abs of control) x100]

ANTIBACTERIAL ACTIVITY:

Test culture:

For the detection of antibacterial activity against gram- positive bacteria, the microbial cultures of *Staphylococcus epidermidis, Bacillus atrophaeus* were obtained respectively from Saffron Pharmaceuticals Industry Faisalabad. Microbial cultures were preserved on agar slants nutrients in tightly sealed bottles at 4 $^{\circ}$C. The cultures were regularly stocked and checked for viability and purity. Test culture was prepared by inoculating the microbes from stock nutrient broth culture and incubated at 37 ± 10 °C for 24 hours.

Detection of antibacterial activity:

The antimicrobial efficacy of the plant extract was evaluated by paper disc diffusion method. The culture plates were prepared and labeled. Sterile filter paper discs impregnated with plant extract (at a concentration of 100 mg/ml and 200 mg/ml) was applied over each culture plates in a sterile fashion. Plates were kept under laminar flow for 30 minutes for the spread of plant concentrate into the agar which was then incubated at 37 $^{\circ}$C for 24 hours. The antibacterial activity was evidenced by the clear zone around the disc. Ciprofloxacin (40µg/ml) was used as standard antibiotic. Each test was repeated three times, and the bacterial activity was expressed as mean of diameter (mm) of zone of inhibitions produced by the plant extract and the controls.

Determination of the relative percentage inhibition:

Relative percentage inhibition of the test material with respect to standard drug was assessed using the following expression:

Relative percentage inhibition zone of ethanolic extract =100x(Y/Z)

Where: Y= Whole region of inhibition of substance

Z=Whole region of inhibition of the standard drug

The entire areas of the inhibitory zones were considered by using the following formula

Area of inhibitory zone = л r2

Where r is the radius of zone of inhibition.[16]

Statistical Analysis:

All data were expressed as mean ± SD and in percentages by using SPSS v23. T-test was used to compare the means of experimental and standard drug. Values with P< 0.05 were considered

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statistically significant. Ethical approval was taken from the office of the ethical committee for utilization of laboratory animals , Bahauddin Zakariya University, No. EC/10/2018.

RESULTS

Antioxidant activity:

The ethanolic extract of *A.indica* (0.5mM) showed 83.76±0.76 DPPH scavenging activity while the standard drug quercetin (0.5mM) showed 93.21±0.97. The % DPPH neutralizing activity of ethanolic concentrates and standard quercetin is shown in figure 2. The comparison showed statistically significant difference between the two drugs.

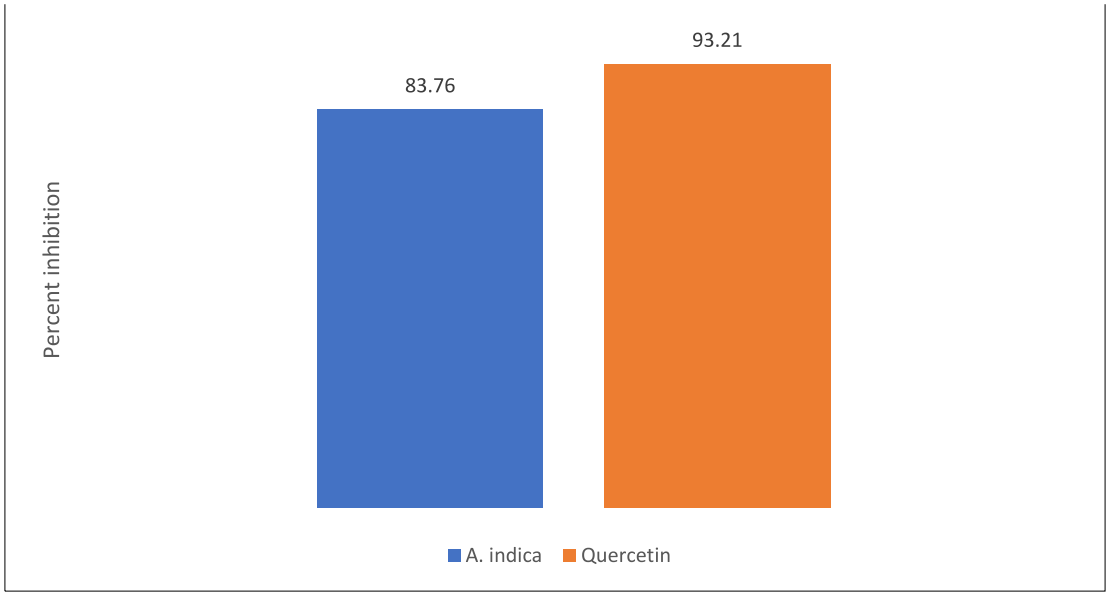

Figure 2: Antioxidant activity (percentage inhibition) of ethanolic extract of *A. indica* & the quercetin

Antibacterial activity:

A.indica (Ai.Cr) showed antibacterial activity against Staphylococcus epidermidis and Bacillus atrophaeus i.e gram (+) bacteria at the dose of 100mg/ml and 200mg/ml. Ciprofloxacin (40µg/ml) is used as a standard drug for Staphylococcus epidermidisSignificant difference of p<0.05 was found when the means (zone of inhibition) of plant extract was compared with the standard drug ciprofloxacin.

Bacterail Strains	Gram Strain (+/-)	*A.indica* Dose (mg/ml)	Zone Of Inhibition (mm/sensitive strain)				Relative Percentage Inhibition	p-value
			A. indica		Standard 40ug/ml			
			Diameter (mm)	Area (mm^2)	Diameter (mm)	Area (mm^2)		
Staphylococcus Epidermidis	+	100mg/ml	16.70	218.9	Ciprofloxacin (20)	314.0	69.7%	p<0.05
Staphylococcus Epidermidis	+	200mg/ml	17.24	233.3	Ciprofloxacin (20)	314.0	74.29%	p<0.05
Bacillus atrophaeus	+	100mg/ml	20.166	319.23	Ciprofloxacin (25)	490.6	65.0%	p<0.05
Bacillus atrophaeus	+	200mg/ml	21.73	370.67	Ciprofloxacin (25)	490.6	75.55%	p<0.05

Table 1: Zone of inhibition (mm) and relative percentage inhibition of *A. indica* & standard drugs

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DISCUSSION

The results of this study showed that A.indica has antioxidant activity. It has significant free radical neutralizing potential. Pal et al., also documented the antioxidant effect of *A.indica* seed extract using various solvents on non-enzymatic hemoglobin glycosylation. The ethanol and chloroform extract exhibited the highest (76.9±0.92 and 70.3±0.45 respective) antioxidant activity at 1 mg/mL concentration. However, the petroleum ether, ethyl acetate, and aqueous extract showed low activity, (45.5±0.53, 62.5±0.35, and 57.1±0.40) respectively.[17] In another activity, the chloroform leaf extract showed 27.62±1.67% and 48.65±1.71% inhibition against nitric oxide and superoxide radicals at the highest (250 µg/mL) concentration.[7] Shahbaz et al. also evaluated the antioxidant potential of seeds of *A. indica.* methanolic seed extract was prepared from the sample and then subjected to a process of sequential fractionation starting from a lower to a higher polarity. The findings indicated very high free radical neutralizing activity of the methanolic extract of *A.indica* seeds and two of its sub fractions prepared with chloroform and ethyl acetate solvents.[18] Another study by Zahoor et al. also indicated that the fruit extracts of *A.indica* have the antioxidant activities that were associated with phenolic compounds of quercetin, hydroxyl benzoic acid, and mandelic acid.[19] Fatima et al., reported significantly altered levels of superoxide dismutase, catalase, malondialdehyde, oxidative stress parameters and myeloperoxidase activity after the treatment with *A.indica.*[20] The flavonoid and tannins detected in the extract might be responsible for its antioxidant activity. These findings are in line with the findings of our study.

A.indica was also found to possess substantial antibacterial effects. In the present study, it showed enhanced antibacterial activity against gram positive organisms. *A.indica* leaves extract was reported to possess a significant antibacterial effect, the methanolic leaves concentrates showed the highest 14±0.5 and 14.5±1 mm zone of inhibition against *Micrococcus luteus* and *Staphylococcus aureus* respectively. The chloroform fraction demonstrated the highest 14.5±0.1 mm inhibition against *Pseudomonas pickettii* and aqueous fractions 16±1, 14±0.5, and 15±0.5 mm against *Bacillus subtilis, Micrococcus luteus,* and *Salmonella Setubal* respectively. Although standard drug cefotaxime showed 33±0, 32±0.01, 31±0.5, 30±0.4 and 30±0.05 mm zone of inhibition against *Staphylococcus aureus, Pseudomonas pickettii, Bacillus subtilis, Micrococcus luteus,* and *Salmonella Setubal* respectively.[21] The lower activity of other (ethyl acetate and methanol) fractions might be due to the low presence of phytochemicals. Other members of this family were also found to have antibacterial properties. In a study, *Aesculus hippocastanum* seeds showed stronger antibacterial action against the Escherichia coli than against Staphylococcus aureus and Streptococcus mutant microbes.[22] A study conducted by Faisal et al. concluded that it also reduces human body's natural response to infection and/or injury by exerting its anti-inflammatory response.[23] Another study documents the bactericidal effect of ZnO nanoparticles using the extract of the shells of Aesculus hippocastanum seeds against B. thuringiensis.[24] Khantwal et al performed a similar study and confirmed the antioxidant and antibacterial activity of *A.indica.* They used methanolic extract of *A.indica* and found enhanced antibacterial activity of the extract against staphylococcus aureus.[25] This study was based on the idea that environmental conditions at different altitudes affect the antioxidant and antibacterial properties of *A. indica* leaves by changing how the plant produces certain natural compounds. But the results revealed that its antioxidant and antibacterial actions are not affected by variying altitudes. Medicinal plants are rich in valuable chemicals and/or metabolites that can treat various diseases in different forms.[26]

CONCLUSION

The present study affirms the fact that *A.indica* possesses substantial level of antioxidant and antibacterial properties, thus making it an effective therapeutic agent in conventional and contemporary medicine.

Limitations

Its a single center study, more studies are needed to generalize the results.

Recommendations

Further pre-clinical studies on different animal models shall be performed to identify its exact antioxidant and antibacterial mechanisms, and to compare its efficacy with standard antioxidant and antibacterial drugs available in the market.

Conflict of interest statement

The authors declare no conflict of interest.

Financial Disclosure Statement

None

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Key Contributions of the Authors	
Author Names	**Author Contributions**
Dr. Aamna Khokhar	A,B,C,D
Dr. Naseer Ahmed	A,B,C,D
Dr. Iram Kamal	A,B,D
Dr. Rizwan Faisal	A,C,D
Afeefah Sattar	B,C,D

Key for Author Contributions:
A.Conception or Design
B.Acquisition, Analysis, or Interpretation of Data
C.Manuscript writing
D.Critical Review and approval
All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved