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EXPLORING THE MEDICINAL POTENTIAL OF SARACA ASOCA (ROXB.) DE WILDE: A COMPREHENSIVE STUDY ON PHYTOCHEMICAL ANALYSIS, ANTIBACTERIAL ACTIVITY, AND ANTIOXIDANT PROPERTIES

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ABSTRACT

Saraca asoca (Roxb.) De Wilde., a globally vulnerable plant species found in the evergreen forests of India, possesses significant medicinal importance. This study focuses on the phytochemical analysis, antibacterial activity, and antioxidant properties of methanol extracts from different parts of Saraca asoca, including bark, flowers, and leaves. Qualitative and quantitative phytochemical analyses revealed the presence of secondary metabolites, such as flavonoids and phenols, with methanol extraction proving most effective. Antibacterial studies demonstrated the efficacy of extracts against both gram-negative and gram-positive bacteria, exhibiting variations in activity levels. Additionally, the antioxidant potential of the bark extract was evaluated using the DPPH assay, showcasing considerable radical scavenging activity. The findings emphasize the plant's potential for pharmaceutical applications and underscore the importance of further research in healthcare-related drug development.

KEYWORDS: *Saraca asoca*, Phytochemical screening, Antibacterial activity, Antioxidant activity, DPPH.

INTRODUCTION

Undoubtedly, plants are a good source of biologically active natural products. While investigating the bioactive natural compounds, it is essential to have access to simple biological tests in order to locate the required activities (Sener et al., 1994). This species, *Saraca asoca*, is currently listed as a 'globally vulnerable' species by the IUCN in 2013. *Saraca asoca* is a medicinally important and globally vulnerable plant species found in the evergreen forests of India (Thakur et al., 1989). India has often been referred to as the medicinal garden of the world, and the medicinal plant *Saraca asoca* has been regarded as one of the foremost plants utilized from antiquity until today. *Saraca asoca* (Roxb.) De Wilde, a small evergreen tree belonging to the family Caesalpiniaceae, is commonly known as *Asoka*, *Sita Asoka*, and *Haempushpam*. It is an evergreen tree that can grow up to 9 meters in height. The flowers are orange-yellow in color and arranged in dense corymbs. It occurs throughout India up to an altitude of 750 meters in the central and eastern Himalayas. The leaves are paripinnate, 15-20 cm long, and the leaflets are 6-12, oblong, and rigidly sub-coriaceous. The leaves are narrowly lanceolate, cork-like at the base, and have a short petistipules that are intra-petiolar and completely united. The bark is dark brown or grey or almost black with a warty surface. The stem bark is rough and uneven due to the presence of rounded or projecting lenticles. The bark is channeled, smooth with circular lenticles, and traversely ridged, sometimes cracked. Fracture splinting exposes striated surface, and a thin whitish and continuous layer is seen beneath the cork leaver. The flowers are fragrant, polygamous apetalous, yellowish-orange turning to scarlet, in short laterally placed corymbose, auxillary panicles, bract small, deciduous, and calyx petaloid. The seeds are 4-8, ellipsoid-oblong, and compressed (Ali et al., 2008 and Jain et al., 1968).

Useful parts of the plant are barks, leaves, flowers and seed. The earliest chronicled mention of this tree is in the Ayurvedic treatise and later in *Charka Samhita* (100 A.D.) in which *Ashoka* has been recommended in formulations for the management of gynecological disorders as anodynes. *Ashokarista* is a very famous formulation from the bark of this plant which is available commercially from various reputed companies and used to treat menstrual disorders. Different parts of *Saraca asoca* plant have been attributed with high medicinal value. *Saraca asoca* bark extracts are often used in Leucorrhea (Shukla et al., 2008). Flowers have shown encouraging anti-ulcer activity in albino rats (Maruthappan et al., 2010). Saracin, a lectin purified from *Saraca indica* seed integument, has been found to agglutinate human lymphocytes and erythrocytes irrespective of the blood group; it causes agglutination of

Ehrlich ascites carcinoma (EAC)3 cells as well as animal erythrocytes (Cibin et al., 2010). Moreover, chemo-preventive activity of flavonoid fraction of Saraca asoca flower was reported in skin carcinogenesis (Ghosh et al., 1999). Larvicidal activity has also been recorded by using Saraca bark and leaves (Methew et al., 2008). Biochemical analyses have shown that leaves of *S. asoca* contain carbohydrates, proteins, glycosides, flavonoids, tannins and saponins (Pradhan et al., 2010). Different plant parts of *S. asoca* provide antibacterial (Nayak et al., 2011) CNS depressant, anti-pyretic, anthelmintic, and analgesic activities (Nayak et al., 2010 and Varma et al., 2010).

All the plant parts are considered to contain medicinal properties. Leaves of *Saraca asoca* are known to contain carbohydrates, proteins, tannins and saponins and show antibacterial activity (Pradhan et al., 2010). Barks and Flowers contain glycosides, steroids, saponins, carbohydrates and tannins (Pal et al., 1985). The flowers are also regarded as a medicinally important plant part and used as a therapeutic agent in the treatment of diabetes, cancer, and hemorrhagic dysentery, uterine infections such as menorrhagia, and other types of uterine disorders. They are also used in bleeding piles and bacillary dysentery. Dried flower buds are reported to have antibacterial activity. An aqueous suspension of Saraca indica flower has anti-ulcer activity in albino rats (Maruthappan et al., 2010). *Saraca asoca* bark and flowers exhibit antitumor activity against DLA, S-180, and Ehrlich ascites carcinoma tumor cell lines, and larvicidal activity has also been recorded (Methew et al., 2008). The chemopreventive activity of the flavonoid fraction of *S. asoca* is reported in skin carcinogenesis. During *'ashoka-sasthi'*, the flower buds are taken orally by women. Though phytoconstituents have been reported earlier in the case of leaves and bark of the plant (Pradhan et al., 2010), no detailed qualitative phytochemical analysis is found for flowers.

The antimicrobial activity of the stem and bark of *Saraca indica* has been evaluated against a standard strain of Staphylococcus aureus, Escherichia coli, and Salmonella typhimurium (Sainath et al., 2009). The leaves of *Saraca indica* have also been evaluated for anthelmintic activity (Nayak et al., 2011), analgesic and antipyretic activities (Pradhan et al., 2010), CNS depressant activity (Yadav et al., 2008). Reports of quantitative estimation of different antioxidants of the bark of *Saraca asoca* are hardly available. The antioxidant property is also related to the condition of the soil and environment where the plant is grown. So in this investigation, we quantitatively estimated different phytochemicals such as total polyphenols, flavonoids, ascorbic acid, and tannins and free radical scavenging activities of DPPH to

evaluate antioxidant properties of the bark of *Saraca asoca.* In the present study, phytochemical analysis, antibacterial activity, and antioxidant study of the different parts of methanol extracts of *Saraca asoca* bark, flower, and leaves were carried out.

MATERIALS AND METHODS

Plant material used for the study included various plant parts (bark, flowers, and leaves) of *S. asoca* collected from the Botanical Garden, Rishikul Ayurvedic College, Haridwar, Uttarakhand. The collected plant materials were identified and voucher specimens were deposited in the medicinal plant museum (Herbarium) of the *Department of Dravyaguna.*

Preparation of Extracts: To prepare the extracts, the plant samples (bark, flowers, and leaves) were washed with distilled water and air-dried at room temperature for 7-10 days. They were then oven-dried at 40°C to remove residual moisture and pulverized. The dried plant parts were stored in air-tight containers for future use. Fifty grams of powdered samples of bark, flowers, and leaves were extracted with methanol by soxhlation method at 60 to 80°C. The three filtrates were separately concentrated in a water bath at 40°C and evaporated under reduced pressure.

Phytochemical Analysis: The four extracts obtained from the powdered flowers of *Saraca asoca* were subjected to phytochemical tests to determine the presence of active secondary metabolites using standard procedures (Mohan et al., 2014).

Antibacterial Activity: The disc diffusion method was used to evaluate the antibacterial activity of the synthesized compounds against four bacterial strains viz; E. coli, P. aeruginosa, K. pneumoniae and S. aureus. Each organism was cultured in nutrient broth at 37°C for 24 h. Then 1% broth culture containing approximately 106 colony forming units (CFU/mL) of test strain was added to nutrient agar medium at 45°C and poured into sterile petri plates. The medium was allowed to solidify. 5 µL of the test compound (40 mg/mL in DMSO) was poured on 4 mm sterile paper discs and placed on nutrient agar plates. In each plate standard antibacterial drug (ampicillin) and metal complexes were added. The plates were incubated at 37°C for 24 h and the antibacterial activity was determined by measuring the diameter of zones showing complete inhibition (mm) (Mohan et al., 2015).

Radical Scavenging Activity: This assay is based on the decrease in the absorbance value of DPPH at 517 nm upon the addition of the complex. The experiment involved diluting the

working solution of the plant extracts and the ascorbic acid standard (700, 600, 500, 400, 300, and 200 µg/µL−1) in methanol. The DPPH concentration was kept constant (2 mL, 0.004 %). To this varying concentration of plant extracts and standard were added. The mixture was shaken vigorously and kept in the dark for 30 min at room temperature. Then the absorbance was measured at 517 nm in a spectrophotometer. The whole experiment was carried out using spectroscopic grade methanol solvent at 298 K. The radical scavenging activity was measured using the following equation: Suppression ratio (%)=[(A0-Ai)/A0)]X 100% (1) where Ai is the absorbance in the presence of the ligand or its complexes, and A0 is the absorbance in the absence of the ligand or its plant extracts.

RESULTS AND DISCUSSION

Phytochemical Analysis: The present study provides valuable information on the bioactive compounds present in *S. asoca*. Qualitative analysis of different parts of the plant (bark, flowers, and leaves) was carried out for alkaloids, flavonoids, glycosides, saponins, phenols, steroids, tannins, and triterpenoids. Methanol, ethanol, and aqueous extracts of the bark, flowers, and leaves contained all the phytochemicals, including flavonoids, glycosides, saponins, phenols, steroids, tannins, and triterpenoids, except for the bark extract, which lacked alkaloids. Additionally, the ethanol extract of flavonoids was absent, and glycosides and tannins were absent in the aqueous extract of the bark. In the flower extract, all phytochemical constituents were present, except alkaloids, which were absent in all extracts, and flavonoids, glycosides, and tannins, which were absent in the aqueous and ethanol extracts of the flower. In the leaves extract, all phytochemical constituents were present, except alkaloids, which were absent in all extracts, steroids, which were absent in the methanolic extract, triterpenoids, which were absent in the ethanolic extract, and glycosides, tannins, and phenols, which were absent in the aqueous extract. These findings are similar to the reports of Saha et al. (2012) and Nayak et al. (2011). The bark, flower, and leaves extracts were quantitatively analyzed for flavonoids and phenols. Interestingly, our study reports the presence of steroids, which was previously reported to be absent in S. asoca (Nayak et al., 2011). Steroids have been attributed to several medicinal properties (Ghatak et al., 2014; Gayathri et al., 2013), making their presence in the plant surprising. However, our study reports the presence of flavonoids and phenols, which agrees with the findings of Ghatak et al. (2015).

Anti-Bacterial Studies: The antibacterial screening of different extracts of *S. asoca* (bark, flower, and leaf) was performed against gram-positive bacteria (*S. aureus)* and gram-negative bacteria (E. coli, P. aeruginosa, and K. pneumonia) by the disk diffusion method. The activities of the compounds were compared with the standard Ampicillin for antibacterial activity. The antibacterial properties of the imine base and its solvent extract were evaluated. The results indicated that the compounds are active in exhibiting antibacterial activity, with bark showing 0.4, 0.2, 0.5; flower showing 0.2, 0.4, 0.6; and leaf showing 0.6, 0.2, 0.2 in gram-negative bacteria, and bark showing 0.6, flower showing 0.4, and leaf showing 0.3 in gram-positive bacteria. The order of activity towards gram-negative bacteria is flower > bark > leaf, and for gram-positive bacteria, the order is bark > flower > leaf. These results are in agreement with the findings of Nayak et al. (2011), Sainth et al. (2009), and Sujatha et al. (2013).

Antioxident Properties: In the present study, the antioxidant properties of the bark extract of *S. asoca* were evaluated by scavenging the stable DPPH radical using a spectrophotometer. The decrease in absorbance at 517 nm was observed as the radical was scavenged by antioxidants, resulting in the reduced form (DPPH-H) and a color change from purple to yellow. The DPPH radical scavenging activities were found to be 28.16% for ascorbic acid and 16.86% for the bark extract at a concentration of 200 µg/µL-1. Ascorbic acid exhibited higher DPPH scavenging activity than the bark extract at all concentrations, with scavenging activities of 95.08% and 78.12% for ascorbic acid and bark extract of S. asoca, respectively, at a concentration of 700 µg/µL-1. The metal scavenging activity, which is a measure of antioxidant property, followed the order of Ascorbic acid > Bark extract of *S. asoca* at a concentration of 200 µg/µL-1, while at a higher concentration, the same order was followed by exchanging their position. These results are similar to the antioxidative potential reported in previous studies (Ghatak et al., 2015; Panchawat et al., 2010).

CONCLUSION

In conclusion, *Saraca asoca* (Roxb.) De Wilde, a globally vulnerable plant species found in the evergreen forests of India, exhibits substantial medicinal importance. This comprehensive study focused on the phytochemical analysis, antibacterial activity, and antioxidant properties of methanol extracts from various parts of *Saraca asoca*, including bark, flowers, and leaves.

The qualitative and quantitative phytochemical analyses uncovered the presence of secondary metabolites, such as flavonoids and phenols, with methanol extraction proving to be the most

effective solvent. Antibacterial studies demonstrated the efficacy of extracts against both gram-negative and gram-positive bacteria, displaying variations in activity levels among different plant parts. Additionally, the antioxidant potential of the bark extract was evaluated using the DPPH assay, revealing significant radical scavenging activity.

The findings underscore the pharmaceutical potential of *Saraca asoca* and highlight its importance in healthcare-related drug development. The diverse medicinal properties observed in this study, including antibacterial and antioxidant activities, emphasize the need for further research and exploration of this plant's therapeutic applications. These insights contribute to the growing body of knowledge on medicinal plants, providing a foundation for the development of drugs in the pharmaceutical industry and emphasizing the significance of continued research in this field.

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