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Assessment of Phenolic and Flavonoid Contents, Antioxidant Properties, and Antimicrobial Activities of *Stocksia Brahuica* Benth

Zahra Ebrahimnezhad[1], Mehdi Dehghani[1*], Hamid Beyzaei[2]

[1]Department of Biology, Faculty of Science, University of Zabol, Zabol, Iran
[2]Department of Chemistry, Faculty of Science, University of Zabol, Zabol, Iran

***Correspondence to**
Mehdi Dehghani, Tel: 09104962810,
Email: dehghanimehdi55@uoz.ac.ir

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Abstract

Introduction: *Stocksia brahuica* Benth. (Sapindaceae) is an invaluable shrubby plant distributed in Iran, Afghanistan, and Balochistan region of Pakistan. In this study, the total phenolic and flavonoid contents and the antioxidant and antimicrobial properties of leaf, fruit, and seed extracts of *S. brahuica* were screened.

Methods: Plant materials were collected in September 2021 from Zahedan, southeast of Iran. The total phenolic and flavonoid contents and total antioxidant capacity were assessed spectrophotometrically using Folin-Ciocalteu, aluminum chloride colorimetric, and DPPH free radical scavenging methods, respectively. The effectiveness of crude hydroethanolic extracts of the leaf, fruit, and seed of *Stocksia brahuica* were also assayed against four gram-negative (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii,* and *Escherichia coli*) and two gram-positive bacteria (*Staphylococcus epidermidis* and *Streptococcus pyogenes*) as well as two fungi (*Aspergillus fumigatus* and *Candida albicans*).

Results: The total phenolic and flavonoid contents were observed to vary from 136.7 to 263.5 mg gallic acid equivalents/g and 26.6 to 104.8 mg quercetin equivalents/g of powdered dry weight. The highest amounts of phenolic and flavonoid content were detected in leaves. Good antioxidant activities were observed with leaves followed by fruits and seeds. Based on our antimicrobial experiments, some plant organs were successful in blocking the growth of tested pathogenic strains.

Conclusion: As a result, natural products derived from this plant can make a significant contribution to the development of the nutritional and pharmaceutical industries.

Keywords: Antimicrobial agent, Antioxidant capacity, Herbal medicine, Natural products, *Stocksia brahuica*

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Introduction

Free radicals are unstable oxygen-containing molecules, atoms, or ions containing an unpaired electron(s), that attack the biological macromolecules such as DNA, lipids, and proteins.[1] Free radicals are made as unavoidable byproducts during normal metabolic reactions in the cells or in the environment.[2] Oxidative stress, resulting from an imbalance between free radicals production and detoxification in cells, is said to be involved directly or indirectly in many pathological conditions for example depression, diabetes mellitus, neurodegenerative disorders, cardiovascular and respiratory diseases, various cancers, and aging.[1,2] However, there must be an equilibrium between oxidants and antioxidants in living organisms and any deviation in this balance can be harmful either as oxidative stress or antioxidative stress.[1,3] Medicinal plants have attracted immense attention due to their high antioxidant capacity. A large amount of evidence has shown that there is a positive relationship between flavonoid and phenolic contents and antioxidant activity of plant organs.[4-6] Also, high amounts of phenolic and flavonoid contents as well as the antioxidant capacity of various plant organs are positively correlated with antibacterial and antifungal properties.

The antibiotic application for the prevention and treatment of infectious diseases in human beings and animals has a long history. Antibiotics have also been extensively used in animal husbandry to foster animal growth and productivity, since additive antibiotics enhance considerably the conversion of food materials into animal products as well as the product quality.[7] However, the extensive use of *antibiotics* in livestock and poultry nutrition, which usually remain in the animal's body, has led to an increase in multidrug-resistant mutants, horizontal gene transfer, and environmental pollution.[8-11] As a result, the European Union (EU) banned the use of veterinary antibiotics in 2006, which initially led to reducing protein products but gradually became less effective.[12] However, plant-derived natural products with high antioxidant and antibiotic properties could serve either as an alternative additive to animal food or a safe way of overcoming antibiotic-resistant bacteria. These plant-based natural compounds offer potentials for safe and qualified antimicrobial agents with fewer adverse effects on final products.

Stocksia Benth. (called KEHTAR in Persian), named after the British physician John Ellertot Stocks, is a monotypic plant genus belonging to the family Sapindaceae.[13] The sole species in this genus, *Stocksia brahuica* Benth., is endemic to Iran, Afghanistan, and Balochistan region of Pakistan.[14,15] *S. brahuica* is a shrubby plant up to 4 m tall with glabrous spiny branches, and sessile, linear, entire leaves. The unisexual flowers bloom in April-May, while the reddish inflated, 3-locular capsules are produced in summer.[15] This shrub can be a good candidate for decorative use in urban areas of arid regions since it shows drought resistance and limited water consumption and provides attractive views.

Much research has been conducted to provide protection against diseases and to design new therapeutic and strengthening drugs. For this purpose, natural products have been extracted from the tissues of various plants and their biological properties have been investigated. The flowers of *S. brahuica* are used traditionally as decoction/infusion in Pakistan to treat stomachaches.[16] However, this environmentally valuable and medicinally unknown endemic plant has attracted very little scientific attention. There is only sparse information on this species in the literature. Apart from the data on taxonomy, only a few Phytochemical studies have been done on the genus *S. brahuica* resulting in the identification of several benzoic acid derivatives, aromatic constituents, stereoisomer, flavone glycosides, Cyanolipids, and tridesmosidic saponins in fruits, seeds and aerial parts of the plant.[17-21] The biological properties of the species have not been evaluated yet. In this study, a population of *S. brahuica* was collected from Zahedan, Iran, and their fruit, leaf, and seed extracts were evaluated for *in vitro* phytochemical characteristics and antioxidant and antimicrobial activities to highlight the biological attributes of this endemic plant and to develop medicines of natural origin.

Materials and Methods

Plant Sample Preparation

Fresh plant materials were harvested from a wild population of *S. brahuica* in ca. 20 km SW Zahedan (2923 N, 6040 E, 1800 m.) during summer 2021 (Figure 1). The plant's scientific name was authenticated by the second author and an herbarium specimen was stored in the Iranian Biological Resource Center Herbarium (IBRC), herbarium code: 3521. The plant organs, including leaves, fruits, and seeds were cleaned, segregated, and air-dried in shade for two weeks. Finally, they were milled into a fine powder using a Moulinex Masterchef blender.

Total Phenolic Content

The Folin-Ciocalteu protocol[22] was used to determine the total phenol content of the plant organs. An amount of 0.5 g of each organ powder was poured into separate test tubes and 1 mL of pure methanol was added to each test tube, followed by putting them into an ultrasonic device for 24 hours. Then the tubes were sealed with parafilm and rested in dark for 24 h. A volume of 0.2 mL of the extracts of seed, fruit, and leaves were measured into test tubes and 2.7 mL distilled water, and 0.1 mL Folin-Ciocalteu reagent were added to each test tube. Aqueous sodium carbonate solution (5 mL, 7% w/v) was added to each mixture after five minutes and incubated for 90 minutes at room temperature. The same procedure was followed for different gallic acid solutions (50, 100, and 200 µg/mL) as standards to produce a calibration curve of absorbance against concentration. The absorbances of the three solutions as well as those of gallic acid solutions were read at 760 nm using a spectrophotometer (model 6405 UV/Vis Jenway). The total phenolic content was expressed as mg gallic acid equivalent (GAE) per gram of extract using the mean of three absorbance readings.

Total Flavonoid Content

The aluminum chloride colorimetric protocol outlined by Chang, Yang et al was used to assess the total flavonoid content of leaves, fruits, and seeds of *S. brahuica*.[23] Quercetin was used as a standard to produce the calibration curve.

Quercetin (2 mg) was dissolved in 70% methanol and three diluted standard solutions of 50, 100, and 200 µg/mL were prepared. These solutions (0.5 mL) were separately mixed with 1.5 mL methanol (70%), 0.1 mL of aluminum chloride (10% w/v), 0.1 mL of potassium acetate (1 M) and 2.8 mL distilled water. The mixtures were kept at room temperature for 40 minutes, prior to measuring their absorbance at 415 nm with a spectrophotometer. The same protocol was pursued to read the absorbance of

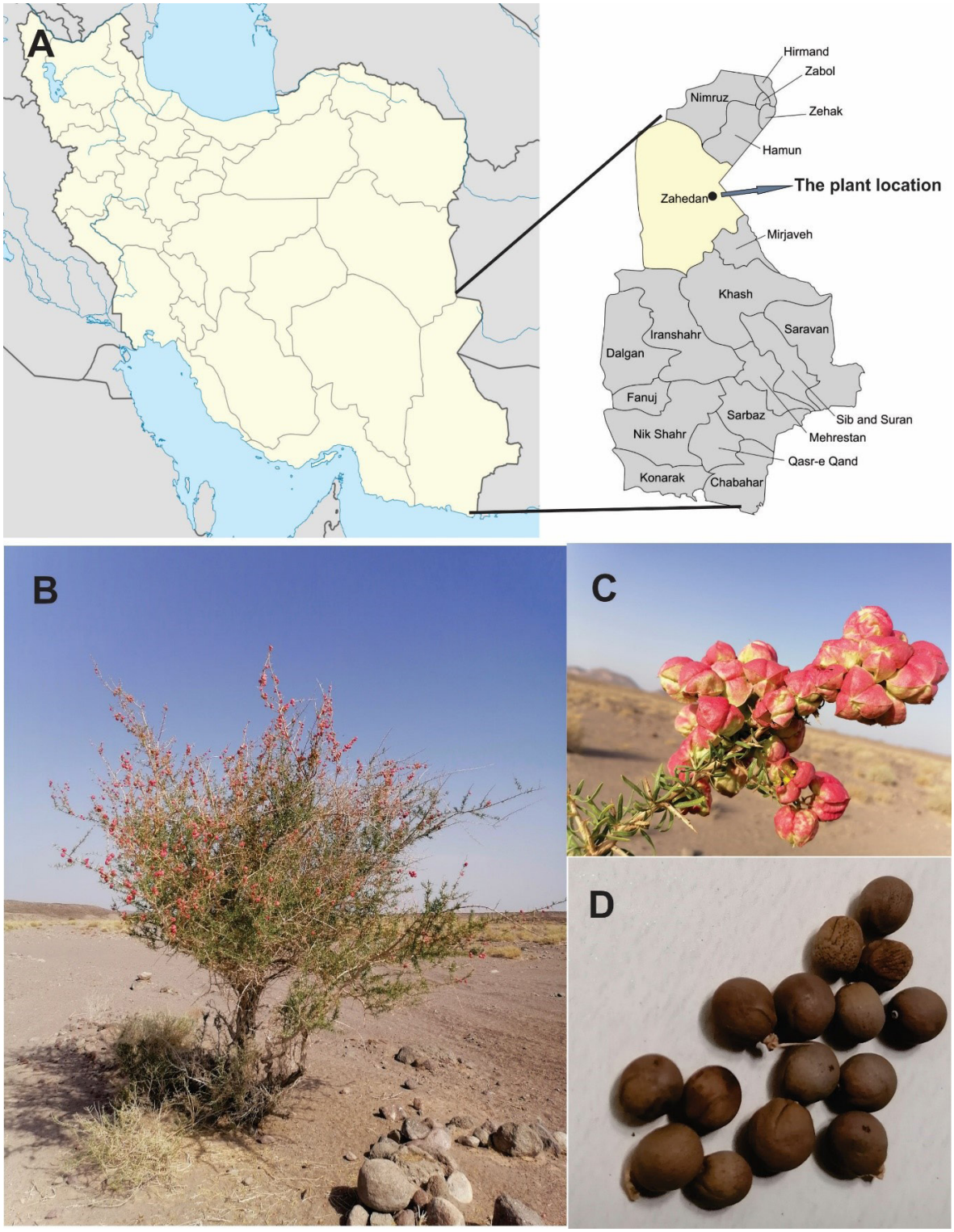

Figure 1. (A) the location of the studied population of *Stocksia brahuica*. (B) the habit and habitat of *Stocksia brahuica*. (C) the inflated three-lobed red capsules. (D) the morphology of the seeds.

methanolic extracts of powdered seeds, fruits, and leaves of *S. brahuica* where 0.5 mL of each extract substituted quercetin in reacting with aluminum chloride using the mean of three absorbance readings. The total flavonoid content was manifested as mg quercetin equivalents (QE) per g dry plant organs.

Antioxidant Activity

The DPPH free radical scavenging method was performed to assess IC_{50} values (half-maximal inhibitory concentration) according to the previously published researches.[24-26] Briefly, solutions containing 1 mL of methanolic extracts of powdered seeds, fruits, and leaves

of *S. brahuica* and 4 ml of newly made DPPH methanolic solution (0.004% w/v) were prepared in separate test tubes. The test tubes remained in blackness for 30 minutes at room temperature. A blank sample was prepared using 4 mL of the DPPH methanolic solution plus 1 mL of methanol. The inhibition percentage (I%) was quantified using the equation: I% = [(A sample −A blank)/(A blank)] × 100 in which *A* stands for absorbance at 517 nm against blank. The same protocol was used for vitamin C as a control to create the calibration curve of absorbance against concentration from which the equation of the straight line was obtained and IC_{50} was computed where *y* equals 50. All tests were repeated three times and the results were expressed as the average of three independent experiments.

Antimicrobial Tests

The hydroethanolic extracts were prepared by the extraction of dried and powdered tissues of *Stocksia brahuica*. Each tissue sample (10 g) was soaked separately in 100 mL EtOH:H_2O (50:50 v/v). The resulting mixtures were shaking in the darkness for 24 hours. Extraction mixtures were filtered through Whatman filter paper to gain the filtrates. The extracts were achieved after solvent evaporation of filtrates in refreshed air, and incubation at 37°C.

Gram-negative bacterial strains including *Pseudomonas aeruginosa* (PTCC 1310, ATCC 10145), *Klebsiella pneumoniae* (PTCC 1290, NCTC 5056), *Acinetobacter baumannii* (PTCC 1855, ATCC BAA-747) and *Escherichia coli* (PTCC 1399, ATCC 25922), gram-positive bacterial strains including *Staphylococcus epidermidis* (PTCC 1435, ATCC 14990) and *Streptococcus pyogenes* (PTCC 1447, ATCC 12204), mold *Aspergillus fumigatus* (PTCC 5009) and yeast *Candida albicans* (PTCC 5027, ATCC 10231) were prepared from the Persian Type Culture Collection (PTCC), Karaj, Iran. Broth microdilution and streak plate methods were performed to determine MIC (minimum inhibitory concentration), MBC (minimum bactericidal concentration) and MFC (minimum fungicidal concentration) according to the M07-A9 and M26-A CLSI (Clinical and Laboratory Standards Institute) guidelines.[27,28] The final concentrations of the extracts ranged from 4096 to 2 µg/mL in each row of 12 wells of a 96-well microplate. Ampicillin and itraconazole were used as positive controls. The results of antimicrobial tests were expressed as the average of three independent experiments.

Results

Total Phenolic and Flavonoid Contents

The total phenolic and flavonoid contents in methanolic extracts of leaf, fruit, and seed of *S. brahuica* were determined according to the Folin-Ciocalteu and aluminum chloride colorimetric methods, sequentially. The equation of the standard curve obtained from various concentrations of gallic acid was Y = 0.0026X-0.0375 (R^2 = 0.9625), based on which the total phenolic content of each plant organ was calculated. In the same way, the total flavonoid content of each plant organ was calculated via the equation of the calibration curve of quercetin as Y = 0.0007X + 0.6165 (R^2 = 0.995). The phenolic and flavonoid contents of various organs of *Stocksia brahuica* have been presented in Table 1. The values ranged from 136.7 to 263.5 mg gallic acid equivalents/g and 26.6 to 104.8 mg quercetin equivalents/g of powdered dry weight, respectively. The highest amounts of phenolic and flavonoid contents were observed in leaves, while the lowest phenolic content was found in seeds. These results indicate that *S. brahuica* is a rich source of phenolic compounds, which candidates it to protect against lipid peroxidation as well as scavenge free radicals.

Scavenging Effects on DPPH Radical

To evaluate the antioxidant potentials of extracts, their H-atom-donating abilities were estimated against DPPH free radicals. The IC_{50} values of the methanolic extracts of different organs of *S. brahuica* have been shown in Table 1. They ranged from 8.3 to 161.9 µg/mL. The highest and the lowest antioxidant activities were found in methanolic extracts of the leaf, and seed of *S. brahuica*, respectively. However, the total antioxidant capacity of *S. brahuica* is lower than that of vitamin C.

Antimicrobial Properties

In vitro antimicrobial susceptibility testing of hydroethanolic extracts of leaf, seed, and fruit of *S. brahuica* were monitored on a wide spectrum of pathogenic fungi and bacteria, as shown in Table 2.

Discussion

The results of our study on the chemical composition, antioxidant capacity, and antimicrobial properties of hydroethanolic extracts of seed, leaf, and fruit of *S. brahuica* indicate that *S. brahuica* is a rich source of phenolic compounds, with a high antioxidant capacity which candidates it to protect against lipid peroxidation as well as scavenge free radicals.

The IC_{50} value, which is calculated from a linear regression analysis, is inversely correlated to the antioxidant activity of a compound.[29,30] The results of the antioxidant assay in this paper suggest the potential benefits of utilization of *Stocksia brahuica* organs

Table 1. Phenolic and Flavonoid Contents and Antioxidant Effects of *Stocksia brahuica* Extracts

Samples	IC_{50} (µg/mL)	Total Phenolic Content mg Gallic Acid/g	Flavonoids Content mg Quercetin/g
Leaf Ex.	8.3	263.5	104.8
Fruit Ex.	25.2	256.5	70.6
Seed Ex.	161.9	136.7	26.6
Vitamin C	3.94	NA	NA

NA, not applicable.

Table 2. Antimicrobial Activity of Extracts

Extracts/ Drugs	Microorganisms											
	P. aeruginosa		*K. pneumoniae*		*A. baumannii*		*E. coli*		*A. fumigatus*		*C. albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
Seed	2048	4096	-	-	2048	4096	-	-	-	-	32	64
Leaf	-	-	-	-	4096	4096	-	-	-	-	512	1024
Fruit	-	-	4096	4096	-	-	2048	4096	2048	4096	512	1024
Ampicillin	1024	2048	32	64	64	128	32	64	-	-	-	-
Itraconazole	-	-	-	-	-	-	-	-	256	512	32	32

MIC, MBC and MFC values were reported as µg/mL.
No inhibitory activity was observed at the highest concentration level (4096 µg/mL) of extracts against both tested Gram-positive strains. Fruit extracts showed a wider range of inhibitory effects than the others. They were the only efficient extracts on *K. pneumoniae*, *E. coli* and *A. fumigatus*. The growth of *P. aeruginosa* strains was inhibited only by seed extracts. All extracts can inhibit the proliferation of *C. albicans* strains; also, the best inhibitory effects were observed on this pathogen.

for human beings and animals. There are significant relationships between the antioxidant properties of compounds and some of their biological activities, such as anti-inflammatory, anti-arthritic, antimicrobial, anti-proliferative, antidiabetic, anti-wrinkle, anti-aging, and neuroprotective. For thousands of years, natural products extracted from herbs have been prescribed for the treatment of diseases caused by the overproduction of reactive oxygen species. Plant phenolic compounds play an essential role in the emergence of these effects.[31] In this study, good antioxidant effects were observed with organs of *S. brahuica* especially its leaf and fruit, coinciding with high levels of phenolic compounds in these organs. Phenolic compounds are widely found in plants and play a vital role in antioxidant activity and destroying free radicals through their hydroxyl groups. Along with phenolic compounds, other structures like terpenes tocopherol and ascorbic acid could develop antioxidant capacity in plant organs both solitarily or synergistically. Moreover, total phenolic compounds and antioxidant properties vary based on the employed solvent, polarity and separation methods. In addition, the antioxidant capacity of phenolic compounds deviates depending on their molecular structure and characteristics.[32,33]

It has been found that a wide spectrum of secondary metabolites produced by medicinal plants such as alkaloids, flavonoids, tannins, and terpenoids have antimicrobial activities.[34] They usually play their antimicrobial roles in one of the following mechanisms.[35]

1) destruction of the bacterial cell membrane; 2) inhibition of bacterial cell-wall biosynthesis; 3) interference in bacterial protein biosynthesis; 4) blocking of a metabolic pathway, and 5) stopping bacterial DNA repair or replication. For example, results obtained from antimicrobial studies of some plant extracts against spoilage and food pathogens indicated the interruption of the cell membrane as their action mechanism.[36] Despite high levels of phenolic contents and good antioxidant activity in various organs of *S. brahuica* especially those of leaves, only a moderate inhibitory effect was observed against *C. albicans*. This contradiction may result from the structure and characteristics of specific phenolic compounds in this plant that exert little antimicrobial effect against tested pathogens. In this study, preliminary *in vitro* screening of antimicrobial effects of hydroethanolic extracts of *S. brahuica* organs was carried out, but there is still much to be known about the phytochemistry and biological properties of this invaluable endemic species.

There is no data on the chemical composition and antimicrobial activity of *Stocksia brahuica* in the literature to be compared with our results. However, a study on phenolic and flavonoid contents and antioxidant activity of its phylogenetically close taxa, *Koelreuteria elegans*, revealed the presence of high levels of phenolic compounds and good antioxidant capacity in leaves, bark, and seeds.[37-39] It should be noted that the results of this paper are the first steps in a long process towards the evaluation and elucidation of other pharmaceutical and biological activities of *S. brahuica*. Unfortunately, there were limitations in this study, for example, it was not possible to investigate various populations of this species growing in different geographical conditions. Also, the results of this study have been obtained *in vitro* and need to be tested *in vivo* as well. Further studies are highly recommended for covering the limitations of this study as well as characterizing bioactive components, biological properties, and potential nutritional and pharmaceutical applications of *S. brahuica*.

Conclusion

The organs of many plants and their extracts and essential oils have been used in folk medicine. In this research, the chemical composition, antioxidant capacity, and antimicrobial properties of hydroethanolic extracts of seed, leaf, and fruit of *S. brahuica* were studied to diversify herbal medicines. Preliminary screening of the phenolic and flavonoid components, antioxidant activity, and antimicrobial activity of extracts of leaf, fruit, and seed of *S. brahuica* indicated the high potential of this endemic shrub for nutrition and pharmaceutical purposes. There is a significant relationship between the antioxidant capacity of compounds and their antidiabetic, anticancer

and anti-neurodegenerative effects. Leaf, fruit, and seed extracts of this species contain considerable amounts of phenols and flavonoids and show good antioxidant activity, suggesting further investigation for isolation of the active components and biological characterizations and medicinal properties of the plant. However, weak to moderate inhibitory effects were observed with extracts against studied pathogens, which may be due to the nature and properties of its phytochemical compounds.

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Authors' Contribution

ZE performed the laboratory work and collected the data. MD was the supervisor of the project and contributed to the project idea and writing the manuscript. HB was the advisor of the project and contributed to providing the equipment for the experiments and writing the paper. All authors have approved the final version of the manuscript.

Competing Interests

The authors declare that there is no conflict of interest.

Ethical Approval

Not applicable.

References

1. Di Meo S, Venditti P. Evolution of the knowledge of free radicals and other oxidants. Oxid Med Cell Longev. 2020;2020:9829176. doi:10.1155/2020/9829176
2. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015;30(1):11-26. doi:10.1007/s12291-014-0446-0
3. Gu T, Wang N, Wu T, Ge Q, Chen L. Antioxidative stress mechanisms behind resveratrol: a multidimensional analysis. J Food Qual. 2021;2021:5571733. doi:10.1155/2021/5571733
4. Mwamatope B, Tembo D, Chikowe I, Kampira E, Nyirenda C. Total phenolic contents and antioxidant activity of *Senna singueana*, *Melia azedarach*, *Moringa oleifera* and *Lannea discolor* herbal plants. Sci Afr. 2020;9:e00481. doi:10.1016/j.sciaf.2020.e00481
5. Abdel-Gawad M, Abdel-Aziz M, El-Sayed M, El-Wakil E, Abdel-Lateef E. In vitro antioxidant, total phenolic and flavonoid contents of six *Allium* species growing in Egypt. J Microbiol Biotechnol Food Sci. 2014;3(4):343-346.
6. Ghafoor K, Al Juhaimi F, Özcan MM, Uslu N, Babiker EE, Mohamed Ahmed IA. Total phenolics, total carotenoids, individual phenolics and antioxidant activity of ginger (*Zingiber officinale*) rhizome as affected by drying methods. LWT. 2020;126:109354. doi:10.1016/j.lwt.2020.109354
7. Hao H, Cheng G, Iqbal Z, et al. Benefits and risks of antimicrobial use in food-producing animals. Front Microbiol. 2014;5:288. doi:10.3389/fmicb.2014.00288
8. Barton MD. Antibiotic use in animal feed and its impact on human health. Nutr Res Rev. 2000;13(2):279-99. doi:10.1079/095442200108729106
9. Gullberg E, Cao S, Berg OG, et al. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog. 2011;7(7):e1002158. doi:10.1371/journal.ppat.1002158
10. Kohanski MA, DePristo MA, Collins JJ. Sublethal antibiotic treatment leads to multidrug resistance via radical-induced mutagenesis. Mol Cell. 2010;37(3):311-320. doi:10.1016/j.molcel.2010.01.003
11. Couce A, Blázquez J. Side effects of antibiotics on genetic variability. FEMS Microbiol Rev. 2009;33(3):531-538. doi:10.1111/j.1574-6976.2009.00165.x
12. Marshall BM, Levy SB. Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev. 2011;24(4):718-733. doi:10.1128/cmr.00002-11
13. Iranshahr M. Introduction to Latin Language and its Botanical Application. Tehran: University of Tehran Press; 2015. [Persian].
14. Abdulia P. Sapindaceae. Flora of West Pakistan, vol. 39, 1-10. Karachi: University of Karachi; 1973.
15. Rechinger KH. Flora Iranica. Sapindaceae. Graz: Akademishe Druck University; 1966.
16. Bibi T, Ahmad M, Bakhsh Tareen R, et al. Ethnobotany of medicinal plants in district Mastung of Balochistan province-Pakistan. J Ethnopharmacol. 2014;157:79-89. doi:10.1016/j.jep.2014.08.042
17. Ahmad VU, Ali Z, Ali MS, Zahid M, Tareen RB. Brahol: a new derivative of allo-inositol from *Stocksia brahuica*. Nat Prod Sci. 1998;4(3):170-173.
18. Ahmad VU, Ali Z, Ali MS, Iqbal F, Zahid M, Tareen RB. Two new aromatic constituents from *Stocksia brahuica*. Zeitschrift für Naturforschung B. 1999;54(7):940-942. doi:10.1515/znb-1999-0718
19. Ahmad VU, Bader S, Arshad S, et al. A new acylated flavone glycoside from the fruits *of Stocksia brauhica*. J Asian Nat Prod Res. 2007;9(3-5):299-305. doi:10.1080/10286020600727327
20. Ahmad VU, Bader S, Arshad S, et al. Brauhenoside A and B: saponins from *Stocksia Brauhica* Benth. Nat Prod Commun. 2008;3(2):1934578X0800300210. doi:10.1177/1934578x0800300210
21. Mikolajczak KL, Smith CR Jr, Tjarks LW. Cyanolipids of *Stocksia brahuica* Benth. seed oil. Biochim Biophys Acta. 1970;210(2):306-314.
22. Sánchez-Rangel JC, Benavides J, Heredia JB, Cisneros-Zevallos L, Jacobo-Velázquez DA. The Folin–Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. Anal Methods. 2013;5(21):5990-5999. doi:10.1039/c3ay41125g
23. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10(3):178-182. doi:10.38212/2224-6614.2748
24. Beyzaei H, Kamali Deljoo M, Aryan R, Ghasemi B, Zahedi MM, Moghaddam-Manesh M. Green multicomponent synthesis, antimicrobial and antioxidant evaluation of novel 5-amino-isoxazole-4-carbonitriles. Chem Cent J. 2018;12(1):114. doi:10.1186/s13065-018-0488-0
25. Beyzaei H, Sargazi S, Bagherzade G, Moradi A, Yarmohammadi E. Ultrasound-assisted synthesis, antioxidant activity and computational study of 1,3,4-oxadiazol-2-amines. Acta Chim Slov. 2021;68(1):109-117. doi:10.17344/acsi.2020.6208
26. Padmaja A, Rajasekhar C, Muralikrishna A, Padmavathi V. Synthesis and antioxidant activity of oxazolyl/thiazolylsulfonylmethyl pyrazoles and isoxazoles. Eur J Med Chem. 2011;46(10):5034-5038. doi:10.1016/j.ejmech.2011.08.010
27. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard, CLSI document

M07-A9. 9th ed. Wayne, Pennsylvania: CLSI; 2012.
28. Clinical and Laboratory Standards Institute (CLSI). Methods for Determining Bactericidal Activity of Antimicrobial Agents. Approved Guideline, CLSI document M26-A. Wayne, Pennsylvania: CLSI; 1998.
29. Semerci AB, İnceçayir D, Konca T, Tunca H, Tunç K. Phenolic constituents, antioxidant and antimicrobial activities of methanolic extracts of some female cones of gymnosperm plant. Indian J Biochem Biophys. 2020;57(3):298-303.
30. Shrivastava A, Aggarwal LM, Mishra SP, Khanna HD, Shahi UP, Pradhan S. Free radicals and antioxidants in normal versus cancerous cells—an overview. Indian J Biochem Biophys. 2019;56(1):7-19.
31. Cosme P, Rodríguez AB, Espino J, Garrido M. Plant phenolics: bioavailability as a key determinant of their potential health-promoting applications. Antioxidants (Basel). 2020;9(12):1263. doi:10.3390/antiox9121263
32. Falah F, Shirani K, Vasiee A, Tabatabaee Yazdi F, Alizadeh Behbahani B. In vitro screening of phytochemicals, antioxidant, antimicrobial, and cytotoxic activity of *Echinops setifer* extract. Biocatal Agric Biotechnol. 2021;35:102102. doi:10.1016/j.bcab.2021.102102
33. Qian J, Hou M, Wu X, Dai C, Sun J, Dong L. A review on the extraction, purification, detection, and pharmacological effects of 2,3,5,4′-tetrahydroxystilbene-2-O-β-d-glucoside from *Polygonum multiflorum*. Biomed Pharmacother. 2020;124:109923. doi:10.1016/j.biopha.2020.109923
34. Álvarez-Martínez FJ, Barrajón-Catalán E, Herranz-López M, Micol V. Antibacterial plant compounds, extracts and essential oils: an updated review on their effects and putative mechanisms of action. Phytomedicine. 2021;90:153626. doi:10.1016/j.phymed.2021.153626
35. Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control. 2019;8:118. doi:10.1186/s13756-019-0559-6
36. Gonelimali FD, Lin J, Miao W, et al. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Front Microbiol. 2018;9:1639. doi:10.3389/fmicb.2018.01639
37. Kumari P, Nehra S, Deen MK. Screening of Chinese rain tree (*Koelreuteria elegans*) leaves, bark and defatted seed cake for study of their total phenolic content, flavonoids and antioxidant properties. J Pharmacogn Phytochem. 2019;8(4):1724-1728.
38. Chase MW, Christenhusz MJM, Fay MF, et al. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot J Linn Soc. 2016;181(1):1-20. doi:10.1111/boj.12385
39. Buerki S, Lowry II PP, Alvarez N, et al. Phylogeny and circumscription of Sapindaceae revisited: molecular sequence data, morphology and biogeography support recognition of a new family, Xanthoceraceae. Plant Ecology and Evolution. 2010; 23;143(2):148-59.