RESEARCH ARTICLE

Antioxidant, Nitric Oxide Scavenging and Antimicrobial Activities of *Bauhinia variegata* And *Sarcostemma acidum*

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Abstract

The demand for discovering alternatives to chemical-based antimicrobial and antioxidant agents that are not only potentially effective but also safe for human consumption and derived from natural sources is steadily increasing. The primary aim of this work is to explore the antioxidant and antibacterial properties of *Bauhinia variegata* (Linn) and *Sarcostemma acidum* (Roxb.) from the leaf and stem extracts, respectively, as well as to quantify their tannin and phenolic content. To assess potential antioxidant attributes, the extract samples were evaluated for their capacity to scavenge radicals of nitric oxide (NO). Additionally, the antibacterial efficacy of these extracts was investigated against two distinct types of bacteria, i.e., gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) using different concentrations. Both the leaf extract of *B. variegata* and the stem extract of *S. acidum* exhibited notable antibacterial activity against the tested microorganisms. Furthermore, the results indicate that these plant extracts possess excellent natural antioxidant and antibacterial properties, suggesting their suitability for applications in materials requiring antioxidant and antibacterial attributes while ensuring safety.

Keywords: Antioxidant, Antibacterial, *Bauhinia variegata*, *Sarcostemma acidum*, Free radial scavenging activities.

Highlights

- *Bauhinia variegata* and *Sarcostemma acidum* are discussed as medicinal plants in Ayurveda.
- Both of these are utilized for therapeutic properties to prevent, alleviate, or cure various health conditions naturally.
- Nitric oxide free radical scavenging assays lie in their ability to measure the antioxidant capacity of a substance, indicating its potential efficacy in neutralizing harmful free radicals.
- Antioxidant properties of *B. variegata and S. acidum* possess significant antioxidant properties, which help neutralize free radicals and protect the body against oxidative stress.
- Both these plants exhibit antimicrobial properties, effectively inhibiting the growth of various pathogenic microorganisms.

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Introduction

Bauhinia variegata L. (Leguminosae family); commonly recognized as mountain ebony or Kachnar in Hindi and Sanskrit; is a deciduous tree. It is widely distributed across India, including the Himalayan region, and thrives in numerous tropical and warm areas globally Khan *et al.* (2019); Ghaisas *et al.* (2009). This plant is just one of more than 200 species within the Bauhinia genus, encompassing trees, shrubs, and vines celebrated for their decorative foliage and vibrant flowers. Typically, a medium-sized tree, *B. variegata* flourishes in partial shade or full sunlight, and propagation is easily accomplished through seed cultivation and air layering. Characterized by deeply cordate, sub-coriaceous leaves measuring 10 to 15 cm in both length and breadth, it produces sizable, aromatic flowers that are purple or whitish, typically emerging when the tree lacks foliage. Its pods are rigid, flat, dehiscent, and measure 15 to 30 cm in length by 1.8 to 2.5 cm in width, typically containing 10 to 15 seeds Joshi *et al.* (2017); Dabur *et al.* (2007). This plant historically has a use in traditional medicine to treat various ailments, and researchers have conducted pharmacological investigations to substantiate its therapeutic potential. The research aims to explore comprehensive information about its traditional applications, theragnostic studies, phytochemical properties, and the pharmacological inquiries conducted on the plant. The bark of *B. variegata* has a grayish-brown exterior and pale-pink interior, characterized by a rugged external surface with longitudinal cracks and fissures. Various parts of the plant, such as the bark, leaves, flowers, and pods, have been employed in traditional medicinal systems for their curative qualities. Researchers have conducted pharmacological studies to assess the plant's effectiveness in treating various conditions Khan *et al.* (2019); Ghaisas *et al.* (2009); Joshi *et al.* (2017); Dabur *et al.* (2007). Phytochemical analyses have unveiled the occurrence of saponins, flavonoids, alkaloids, tannins, and other compounds within the plant. Pharmacological investigations have elucidated the plant's potential as an antioxidant, anti-inflammatory, anti-diabetic, anti-tumor, and hepatic-protective

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agent. In summary, *B. variegata* is a plant renowned for its substantial medicinal attributes, historically employed to address a diverse array of ailments Parekh *et al.* (2006); Dos Santos *et al.* (2018); Dhonde *et al.* (2007); Pokhrel *et al.* (2002); Rajkapoor *et al.* (2003a).

Similarly, *Sarcostemma acidum*, a popular medicinal plant indigenous to India, has been identified as a contender for the "Soma" plant by various authors. According to Aryan traditions, "Soma" (or "Somlata") was used to prepare a rejuvenating drink called "Somras" Rajkapoor *et al.* (2003b); Pandey (2018). The original source of the "Soma" plant has remained a mystery for over two and a half centuries and continues to be a topic of debate among Vedic scholars and botanists. This plant is widely distributed across various regions of India, Pakistan, and Europe. In India, this plant is typically found in arid rocky areas in states such as Bengal, Deccan, Bihar, Konkan, Tamil Nadu, Kerala, Madhya Pradesh (M.P.), and Maharashtra Pandey (2018); Dev *et al.* (2017); Dave *et al.* (2014). *S. acidum* boasts an array of medicinal properties, being characterized as cooling, alternate, bitter, acrid, narcotic, antiviral, emetic, and rejuvenating. It has historically been employed for numerous medicinal purposes in different regions of India Kalmath *et al.* (2012); Venma *et al.* (2002); Gulshan *et al.* (2017); Mallam *et al.* (2012); Vishwakarma *et al.* (2019); Madhavan and Tharakan (2020). For instance, in the Bidhar district of Karnataka, the plant's latex is topically applied to wounds and cuts. In the Sirumalai Hills of Andhra Pradesh, a mixture of three drops of the plant's latex with honey is consumed orally three times a day to alleviate chronic ulcers. In cases of infants suffering from earaches, a dry powder derived from the plant is prepared as a decoction and administered. Lactating mothers in the Purulia district of West Bengal are advised to consume the milky latex for medicinal purposes. Furthermore, the plant's stem is utilized in Madhya Pradesh to treat bone fractures, while a mixture of stem juice and water is given to individuals afflicted with rheumatism, arthritis, and joint pain. In the Nimar area of M. P., a dry powder combined with mustard oil is externally applied to relieve earaches. Among the ethnic communities in Krishna district, Andhra Pradesh, the milky latex is used as a lotion and as a method to combat white ants (termites). The plant's roots are employed for snakebite cases, while an infusion is ingested in instances of dog bites. Additionally, the dry stem serves as an emetic in the Thar desert. These pharmacological investigations conducted on both of these plants have corroborated their efficacy and shed light on the mechanisms of action of their bioactive compounds. It is crucial to conduct additional research in order to acquire a thorough comprehension of the therapeutic capabilities of these plants and to formulate medications that are both effective and safe for treating a diverse array of illnesses. Hence, researchers should continue their efforts to create effective and safe medications utilizing these plants.

This research seeks to present the antioxidant and antibacterial properties of *B. variegata* leaf extracts (BVLE) and *S. acidum* stem extracts (SASE) through an analysis of their *NO* Free Radical Scavenging Activity. The subsequent sections of this paper are organized as follows: Sections II and III detail the materials and methodology and the results of the study, respectively. Finally, a conclusion is drawn in Section IV.

Material and Methods

The material preparation and the method used for analyzing the antioxidant and antibacterial properties of BVLE and SASE is presented in this section as follows.

Sample Preparation

In April 2021, the specimens of BVLE and SASE were gathered from Newtown, Kolkata, India (22.5754°N latitude and 88.4798°E longitude) as depicted in Fig. 1. These specimens underwent expert identification by the Center for Microbiology and Bio-Technology (CMBT) research and training institute based in Bhopal, Madhya Pradesh, India. Fig. 2 (a) and (b) provide the representations of the green plant in step I, shaded dried in step II and ground samples of BVLE and SASE in Step III, respectively. The samples were manually ground, and subsequently, the machine-ground leaves and stems (amounting to 250 grams) were subjected to extraction using ethanol solvent at room temperature (25 ± 2°C). The resultant sample was then thickened by evaporating the solvent under reduced pressure and at 40°C utilizing a rotary evaporator, ultimately yielding a concentrated, gum-like substance with a dark green hue.

TPC and TFC calculations

Phytochemical screening plays a pivotal role in the identification of chemical compounds within plants, with the isolated

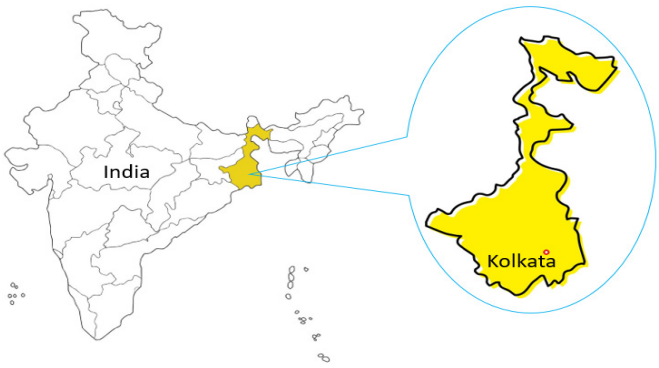


Fig. 1: Location of the Sample Collection

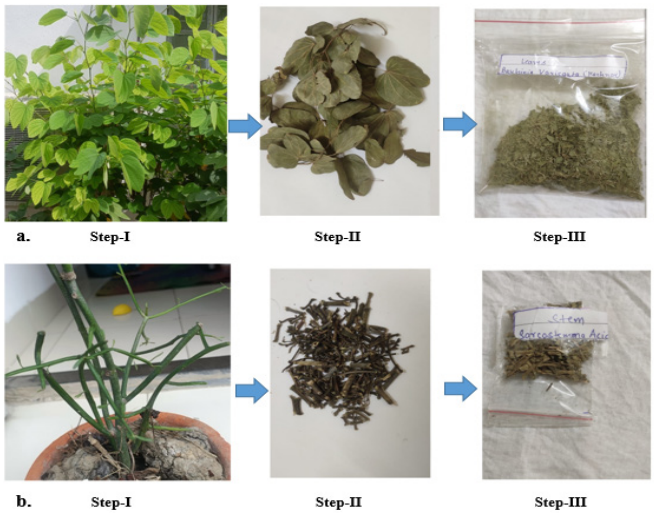


Fig. 2: Step-wise Sample Preparation of a) BVLE; and b) SASE

compounds often serving diverse pharmacological purposes. In this particular study, we performed phytochemical screening on ethanolic plant extracts to confirm the presence of flavonoids and tannins, both of which have significant medicinal importance, as highlighted by Razali *et al.* (2008). Tannins exhibit astringent and antimicrobial properties, while the content of flavonoids is a crucial indicator of the overall antioxidant activity of plants, as noted by Javanmardi *et al.* (2003); Li *et al.* (2009). The presence of these varied chemical constituents directly impacts the therapeutic and pharmacological qualities of medicinal plants. Therefore, the investigation of the chemical composition of plant materials is directly linked to their local medicinal applications.

In this study, freshly prepared ethanolic extracts from these plant sources underwent qualitative analysis to detect specific chemical components. The phytochemical analysis of the extracts involved the use of various reagents and chemicals, as follows: magnesium and hydrochloric acid (HCl) were employed to detect flavonoids, while ferric chloride and potassium dichromate solutions were used to identify tannins. These components were identified based on characteristic color changes, following established protocols as outlined by Evans (2009).

Total Flavonoid Content (TFC) Estimation

The quantification of flavonoid content in the isolated crude extracts (BVLE and SASE) was carried out following a specific procedure detailed in Fattahi *et al.* (2014). To begin, take 0.5 mL of the sample (the extract) and combine it with 1.25 mL of distilled water in a clean test-tube. Following that, introduce 0.075 mL of a 5% sodium nitrite solution and let the combination still for 5 minutes. After this period, introduce 0.15 mL of a 10% aluminum chloride solution. After a 6-minute interval, add 0.5 mL of 1.0 M sodium hydroxide and subsequently mix the solution with an additional 0.275 mL of distilled water. Immediately measure the absorbance of the resultant mixture at a wavelength of 510 nm. Quercetin served as the standard antioxidant, and its efficacy exhibited concentration-dependent behavior, as depicted in Fig. 3. The total flavonoid concentration (TFC) was quantified in milligrams of quercetin equivalent (QE) per gram of dry weight.

Total Phenolic Content (TPC) Estimation

The determination of total phenolic content involved a modified Folin-Ciocalteau method. The assessment of total phenolic content in the isolated crude (BVLE and SASE) followed the procedure described in Gulcin (2020). To initiate the process, we combined 1.0 mL of the sample with the same amount of Folin and Ciocalteu's phenol reagent. After a 3-minute interval, we introduced 1.0 mL of saturated sodium carbonate (Na_2CO_3, approximately 35%) into the mixture and adjusted the absolute volume to 10 mL with distilled water. The reaction was then allowed to proceed in darkness for 90 minutes, and subsequent analysis was performed using a UV-Vis spectrophotometer at an absorbance of 760 nm. Tannic acid was used as the standard, with concentrations ranging from 200 to 1000 ppm. Similarly, tannic acid was employed as the standard antioxidant, and its activity exhibited a concentration-dependent relationship, as illustrated in the standard calibration curve in Fig. 4. The total phenolic content was quantified in milligrams of tannic acid equivalent (TAE) per gram of dry weight.

Nitric Oxide Scavenging

To assess the antioxidant activities of both extracts, we employed the nitric oxide scavenging assay described by *Nakai K. et al.* (2021). In this procedure, we prepared the extracts from a 10 mg/mL ethanol crude extract. Subsequently, these extracts underwent a series of dilutions with distilled water, resulting in concentrations ranging from 100 to 1000 µg/mL for both the plant extract and the standard ascorbic acid. These diluted solutions were then stored at 4°C for future use.

To Prepare Griess reagent (GR), $C_6H_8N_2O_2S$ (sulphanilamide), H_3PO_4 (phosphoric acid), $C_{12}H_{14}N_2.2ClH$ (N-(1-Naphthyl), $Na_2[Fe(CN)_5NO]$ (sodium nitroprusside), Phosphate buffered saline (PBS) are a basic chemical requirement. The GR was formulated by combining an equal amount of 1% $C_6H_8N_2O_2S$ in 2.5% H_3PO_4 and 0.1% $C_{12}H_{14}N_2.2ClH$ in 2.5% H_3PO_4 just before its application. An amount of 0.5 mL of 10 mM $Na_2[Fe(CN)_5NO]$ in PBS was combined with 1-mL of the various concentrations of ethanol extracts (starting from 100–1000 µg/mL) and permitted to undergo incubation at 25°C for 180 minutes. Following the incubation period, the extract was blended with a freshly prepared GR

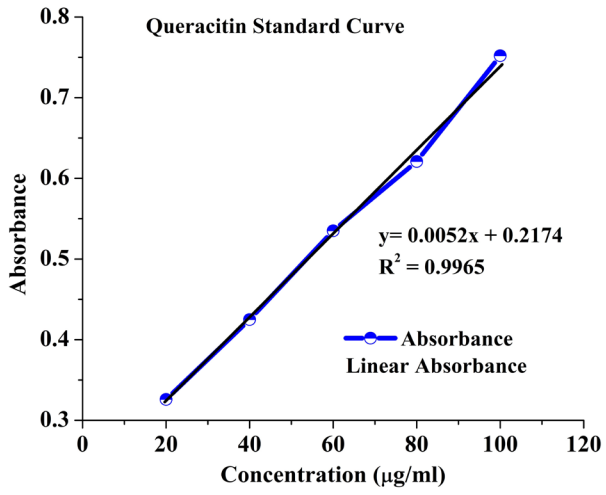


Fig. 3: Calibration graph of Queracitin

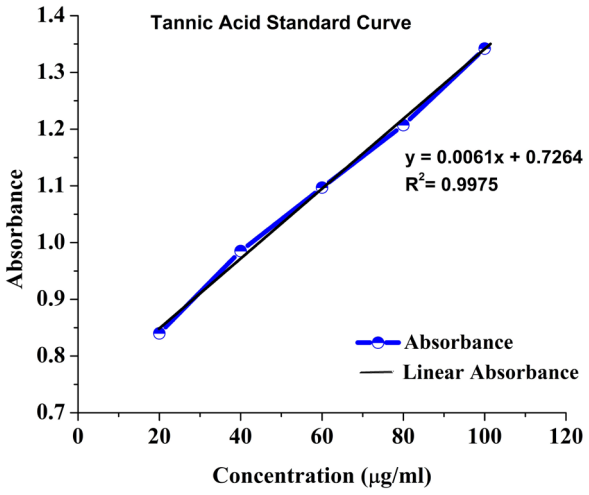


Fig. 4: Calibration graph of Tannic Acid

in equal volume. Control samples, devoid of extracts but containing an equal quantity of buffer, were prepared using the same procedure as the test samples. Furthermore, there were color tubes containing ethanol extracts at identical concentrations, excluding $Na_2[Fe(CN)_5NO]$.

Subsequently, 150 µL of the resulting reaction mixture was reassigned to a 96-well plate, and the absorbance was measured at 546 nm. Ascorbic acid served as the positive control, and the inhibition values were examined and noted for both the extract and the standard. The percentage of nitrite radical scavenging activity of the ethanol extracts was also assessed using the formula provided below:

Nitric Oxide Scavenged (%) = [1-(Absorbance of Sample)/ (Absorbance of Control)] × 100

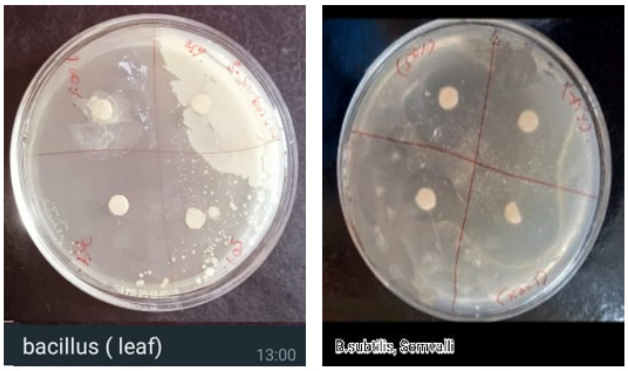
It is important to mention here that the above test was performed with both the extracts separately.

Antimicrobial Activity

To evaluate the antibacterial effectiveness, the isolation of various microbial samples from different oral flora using the swab technique is done. Subsequently, these samples were spread onto specific culture plates and incubated at a temperature of 37°C as shown in Figs 5-8. After the incubation period, the bacterial isolates underwent gram staining and were examined under a compound microscope at a magnification of 100X. Based on their morphological characteristics, we identified four distinct bacterial isolates: *E. coli, P. aeruginosa*, *B. subtilis*, and *S. aureus*, which belonged to both the gram-positive (GP) and gram-negative (GN) categories. To assess the antibacterial properties of these extracts against these four bacterial species, we utilized the disc diffusion method, as outlined in *Zaki AH et al.* (2024). In this method, a filter paper disc saturated with a chemical compound was placed onto an agar medium. The chemical then diffused from the disc into the surrounding agar, establishing a region of chemical infiltration limited to the vicinity around the disc. The solubility and molecular size of the chemical determined the size of this infiltration zone. If an organism was introduced onto the agar, it would not proliferate in the proximity of the disc if it was vulnerable to the chemical. This absence of development encircling the disc is denoted as a "zone of inhibition." After incubation, we measured the diameter of this zone to the nearest whole millimeter at the point where there was a clear reduction of 80% in bacterial growth.

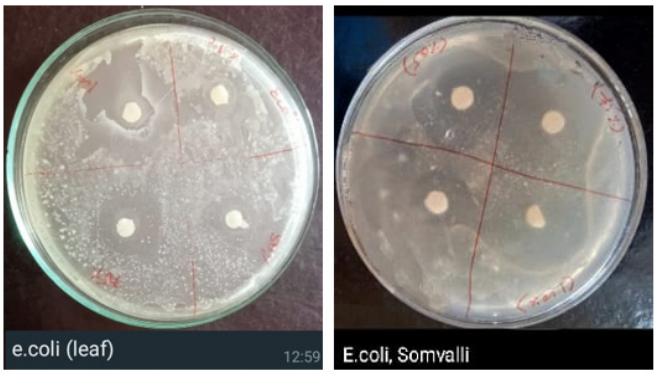
Results and Discussion

The TPC of the plant samples was calculated through Folin-Ciocalteu's reagent and stated in tannic acid equivalent, utilizing the following standard equation: y = 0.0061x+ 0.7264, with an R^2 value of 0.9975. The TPC in the ethanol extracts of BVLE and SASE was quantified as 20.87 and 19.83 mg TA/g, respectively.



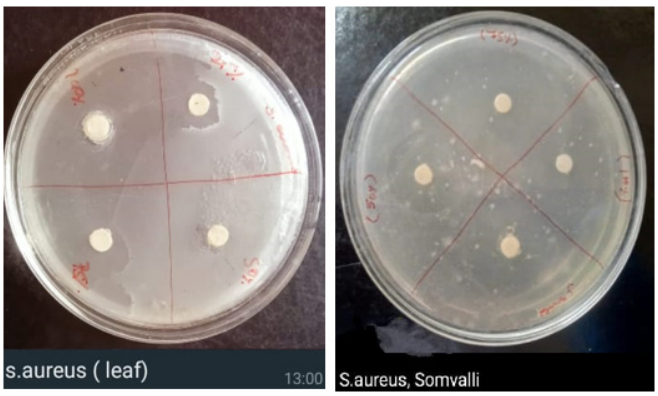
(a) (b)

Fig. 5: Activity of; (a) BVLE; and (b) SASE; against Bacillus cereus



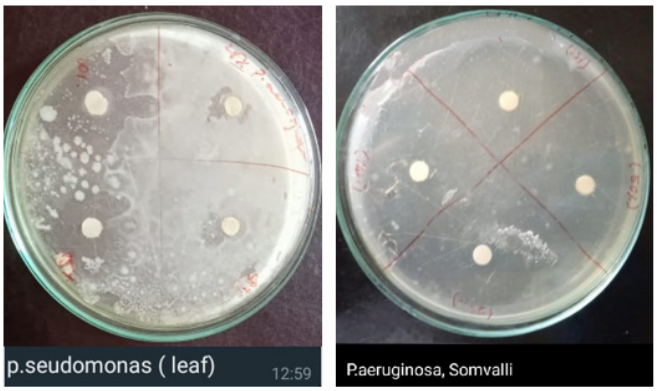
(a) (b)

Fig. 7: Activity of; (a) BVLE; and (b) SASE; against Escherichia coli



(a) (b)

Fig. 6: Activity of; (a) BVLE; and (b) SASE; against Staphylococcus aureus



(a) (b)

Fig. 8: Activity of; (a) BVLE; and (b) SASE; against Pseudomonas aeruginosa

Likewise, the quantity of flavonoid was expressed as quercetin equivalent, employing the standard curve equation: y = 0.0052x + 0.2174, with an R^2 value of 0.9965, measured in milligrams of quercetin per gram of extract. The BVLE exhibited a flavonoid concentration of 18.79 mg/g, while the SASE had a concentration of 22.38 mg/g. These values are also presented in Table 1.

In the nitric oxide (*NO*) radical scavenging assay, the results demonstrate a significant resemblance to the standard ascorbic acid. In the *NO* scavenging method, the ethanol BVLE displayed antioxidant activity against free radicals at varying concentrations. Specifically, at 20, 40, 60, 80, and 100 mg/mL, the scavenging percentages were found to be 8.84, 28.1, 41.17, 57.68, and 82.45%, respectively, as illustrated in Fig. 9. Similarly, the SASE exhibited inhibition of the *NO* radical at percentages of 23, 32.7, 38.1, 42.52, and 48.15% at the same concentrations, as depicted in Fig. 10. The capacity to scavenge the free radical, NO, was assessed at an absorbance of 517 nm. The IC_{50} values for the ethanol extracts of BVLE and SASE were determined to be 31.36 ± 0.048 and 38.96 ± 0.076 µg/mL, respectively, whereas ascorbic acid, used as a reference, exhibited an IC_{50} value of 3.125 ± 0.052 µg/mL. These findings indicate that the extracts derived from BVLE and SASE possess the ability to combat various free radicals in different biological systems, suggesting their potential as valuable therapeutic agents for mitigating damage caused by radical-induced pathological processes.

In the analysis of antimicrobial activities, four distinct microorganisms, namely *B. cereus, S. aureus, E. coli* and *P. aeruginosa* were chosen to investigate the antibacterial mechanisms of both the plant sample extracts. At a concentration of 20% (w/v), all the lab results of samples displayed the antibacterial activity. As a result, by using the agar well diffusion method, the same concentration was subsequently utilized to establish their minimum inhibitory concentrations. Additionally, it was utilized to assess their usefulness in regulating foodborne pathogens and decomposed microbes, as detailed in Mostafa *et al.* (2018). The assessment of antibacterial activity for both plant extracts can be observed in Figs 11 and 12, respectively. The outcomes indicated that both the sample extracts demonstrated potential effectiveness in inhibiting microbial development, albeit with unpredictable potency. Among the tested pathogenic bacteria, the extract derived from BVLE exhibited the highest efficacy in inhibiting the growth of *S.*

Table 1: TPC and TFC analysis in BVLE and SASE

Plant extract	*TPC (mg/g)*	*TFC (mg/g)*
BVLE	20.87	18.79
SASE	19.83	22.38

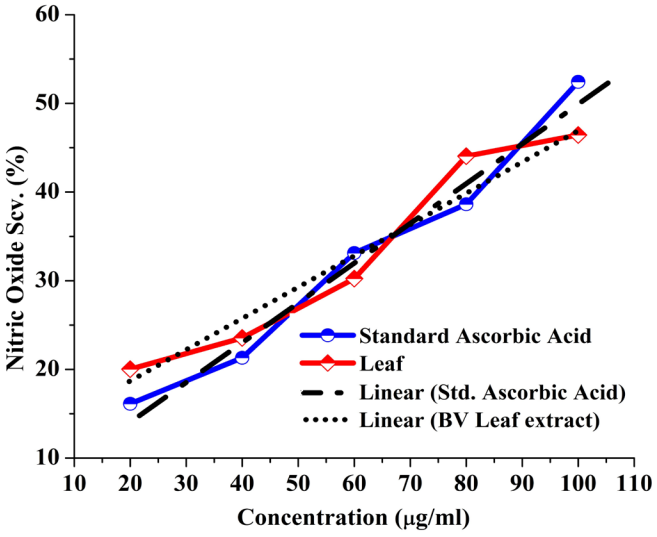


Fig. 9: Nitric Oxide Scavenging Activity of BVLE

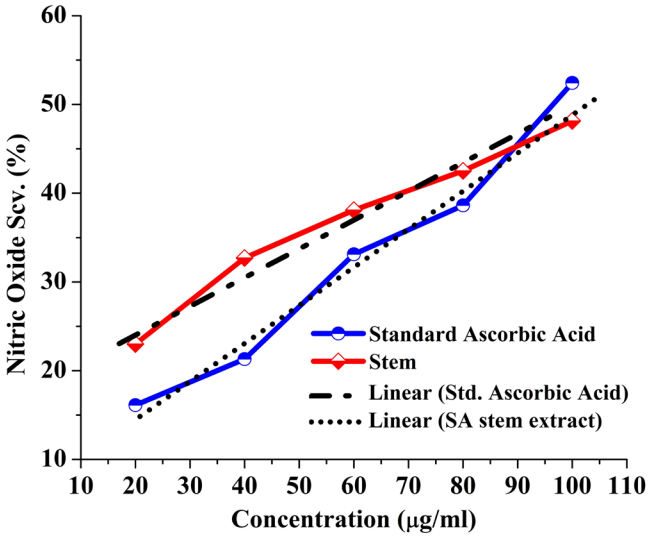


Fig. 10: Nitric Oxide Scavenging Activity of SASE

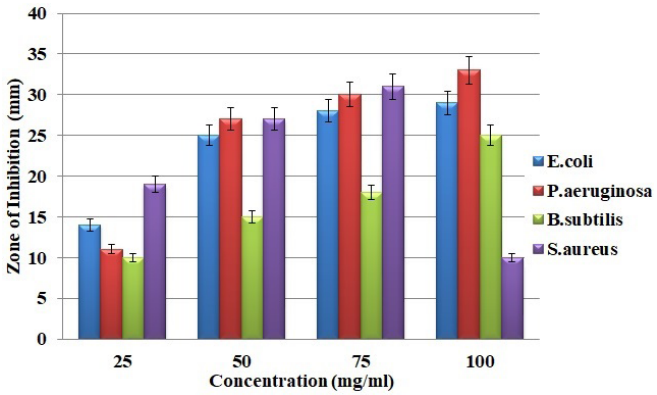


Fig. 11: Antibacterial activity of BVLE

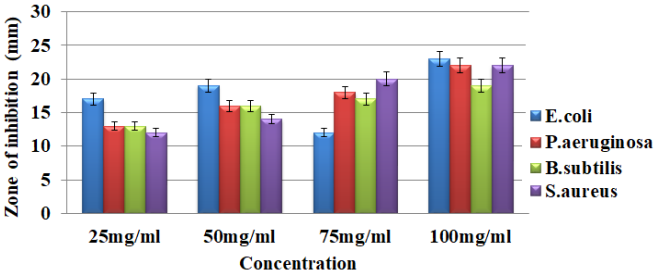


Fig. 12: Antibacterial activity of SASE

aureus at a concentration of 25 mg/mL. Conversely, the SASE displayed the most significant effectiveness against *E. coli* at the same concentration. However, at a concentration of 50 mg/mL, the BVLE displayed the most pronounced inhibition of *E. coli, P. aeruginosa*, and *S. aureus* growth among the tested pathogenic bacteria, while the SASE exhibited enhanced performance, particularly against *E. coli*. At a concentration of 75 mg/mL, the BVLE showed moderate effectiveness in inhibiting *B. subtilis* growth among the tested pathogenic bacteria, while the SASE demonstrated considerable efficacy against all microorganisms except *E. coli* (which showed comparatively lower inhibition) at the same concentration. Finally, at a concentration of 100 mg/mL, the BVLE displayed relatively lower effectiveness in retarding *S. aureus* growth among the tested pathogenic bacteria, whereas the SASE exhibited comparable effectiveness against all microorganisms at the same concentration.

Conclusion

The research results emphasize the bright prospects of *B. variegata* leaf extracts (BVLE) and *S. acidum* stem extracts (SASE) as natural substitutes for synthetic preservatives. This study has successfully conducted and demonstrated nitric oxide scavenging activity, TPC, TFC analysis, and antibacterial activity. The results represent that both the BVLE and the SASE exhibit remarkable antioxidant and antibacterial characteristics, suggesting their potential utility in natural medicines, preservatives, and various other suitable applications.

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Authors' Contribution

"NS" performed the experiments and write the manuscript. "SKM" and "SD" guided during research and done the final correction of the updated version of the manuscript.

Conflict Of Interest

The authors state no conflict of interest.

References

Dabur, R., Gupta, A., Mandal, T. K., Singh, D. D., Bajpai, V., Gurav, A. M., and Lavekar, G. S. (2007). Antimicrobial activity of some Indian medicinal plants. African Journal of Traditional, Complementary and Alternative Medicines, 4(3), 313-318.

Dave, B. K., Dhirawat, R., Kumawat, M. (2014). Pharmacognostical study of a medicinal plant of india–*Sarcostemma acidum*. *Int J Pharm Phytochem Res*, 6:690–7. at http://impactfactor.org/PDF /IJPPR/6/IJPPR, Vol6,Issue4,Article4.pdf

Dev, S. K., Sharma, M., Srivastava, R., Choudhury, P. K. (2017). Phytochemical and pharmacological aspects of *Sarcostemma acidum* (roxb.) voigt. *Journal of Pharmacy Research*, 11(11):1429-31. Available online at echarak.in/echarak/templates /Sarcostemma% 20acidum%20(Roxb.)%20Voigt.%20.pdf

Dhonde, S., Siraskar, B., Kulkarni, A., Kullarni, A., and Bingi, S. (2007). Haematinic activity of ethanolic extract of stem bark of *Bauhinia variegata* linn. *Int J Green Pharm*, 1(3-4):28–33. doi: 10.4103/ijp.IJP 107 16.

Dos Santos, M. C., Kroetz, T., Dora, C. L., Giacomelli, F. C., Frizon, T. E. A., Pich, C. T., da Silva Pinto, L., Soares, A. S., Rodembusch, F. S., de Lima, V. R., *et al.* (2018). Elucidating *Bauhinia variegata* lectin/phosphatidylcholine interactions in lectin-containing liposomes. *Journal of colloid and interface science*, 519:232–241. Dol: 10.1016/j.jcis.2018.02.028.

Evans, W. C. (2009). *Trease and Evans' pharmacognosy*. Elsevier Health Sciences. https://doi.org/10.1016/B978-0-7020-2933-2.00044-7.

Fattahi S, Zabihi E, Abedian Z, Pourbagher R, Motevalizadeh Ardekani A, Mostafazadeh A, Akhavan-Niaki H. (2014). Total Phenolic and Flavonoid Contents of Aqueous Extract of Stinging Nettle and In Vitro Antiproliferative Effect on Hela and BT-474 Cell Lines. Int J Mol Cell Med. Spring;3(2):102-7. PMID: 25035860; PMCID: PMC4082812.

Ghaisas, M., Shaikh, S., and Deshpande, A. (2009). Evaluation of the immunomodulatory activity of ethanolic extract of the stem bark of *Bauhinia variegata* linn. *International Journal of Green Pharmacy (IJGP)*, 3(1). Dol : 10.22377/ijgp.v3i1.60

Nakai, K., and Tsuruta, D. (2021). What are reactive oxygen species, free radicals, and oxidative stress in skin diseases?. International journal of molecular sciences, 22(19), 10799.

Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. Archives of toxicology, 94(3), 651-715. https://doi.org/10.1007/s00204-020-02689-3.

Gulshan, M., Chandrasekhar, G., Kumar, B., and Ramarao, N. (2017). Antiulcer activity of ethanolic *Sarcostemma acidum* stem extract. *Int Res J Pharm*, 8(6):91–94. DOI: 10.7897/2230-8407.086103

Javanmardi, J., Stushnoff, C., Locke, E., and Vivanco, J. (2003). Antioxidant activity and total phenolic content of iranian ocimum accessions. *Food chemistry*, 83(4):547–550. http://dx.doi.org/10.1016/S0308-8146(03)00151-1.

Joshi, V. K., Joshi, A., and Dhiman, K. S. (2017). The Ayurvedic Pharmacopoeia of India, development and perspectives. Journal of ethnopharmacology, 197, 32-38.

Kalmath, S., Patil, M., Kritika, S., Mahantesh, S., and Patil, C. (2012). Existancy and survey of medicinal plants of bidar district, karnataka (india). *World Research Journal of Medicinal and Aromatic Plants*, 1(1):14–21. Available online at http://www.bioinfo.in/contents.php?id=159.

Khan, Z.A.; Siddiqui, M.F.; Park, S. (2019). Current and Emerging Methods of Antibiotic Susceptibility Testing. Diagnostics 9 (49). https://doi.org/10.3390/diagnostics902004.

Madhavan, M. and Tharakan, S. T. (2020). Total phenol quantification and anthelmintic activity of *Sarcostemma acidum* (roxb.) voigt. *Journal of Pharmaceutical Sciences and Research*, 12(1):28–30. Online available at: https://www.jpsr.pharmainfo.in/Documents/Volumes/ vol12issue01 /jpsr12012005.pdf

Mallam, A., Angothu, S., Gurajala, S., and Khuddus, G. A. (2012). Antimicrobial activity of *Sarcostemma acidum* voigt (apocynaceae) stem. *International Journal of Biological and Pharmaceutical Research*, 3(6):752–757. Dol: http://ijbpr.com/doi/MTU0a2FsYWkxNDc4NTIzNjk=

Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., and Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi journal of biological sciences*, 25(2):361–366. Dol: https://doi.org/10.1016/j.sjbs.2017.02.004.

Pandey, S. (2018). Ethnomedicinal potential of *Sarcostemma acidum* in different regions in india. *Asian J Pharm Clin Res*, 11(5):395–400. Dol: https://doi.org/10.22159/ajpcr.2018.v11i5.24887.

Parekh, J., Karathia, N., and Chanda, S. (2006). Screening of some traditionally used medicinal plants for potential antibacterial activity. *Indian Journal of Pharmaceutical Sciences*, 68(6). Dol: 10.4103/0250-474X.31031.

Pokhrel, N. R., Adhikari, R., and Baral, M. (2002). In-vitro evaluation of the antimicrobial activity of *Bauhinia variegata*, locally known as koiralo. *World Journal of Microbiology and Biotechnology*, 18:69–71. DOI:10.1023/A:1013969628634

Rajkapoor, B., Jayakar, B., Anandan, R., and Kavimani, S. (2003a). Anti-ulcer effect of *Bauhinia variegata* linn. in rats. *Journal of natural remedies*, pages 215–217. Dol: 10.18311/jnr/2003/170

Rajkapoor, B., Jayakar, B., and Murugesh, N. (2003b). Antitumour activity

of *Bauhinia variegata* on dalton's ascitic lymphoma. *Journal of Ethnopharmacology*, 89(1):107–109. DOI: 10.1016/s0378-8741(03)00264-2.

Razali, N., Razab, R., Junit, S. M., and Aziz, A. A. (2008). Radical scavenging and reducing properties of extracts of cashew shoots (anacardium occidentale). *Food chemistry*, 111(1):38–44. DoI: https://doi.org/10.1016/j.foodchem.2008.03.024.

Venma, P. K., Sharma, A., Mathur, A., Sharma, P., Gupta, R., Joshi, S., and Dixit, V. (2002). Effect of *Sarcostemma acidum* stem extract on spermatogenesis in male albino rats. *Asian journal of andrology*, 4(1):43–47. Available online at https://pubmed.ncbi.nlm.nih.gov/11907627/

Vishwakarma, R. K., Yadav, A., and Jain, A. P. (2019). Phytochemical and pharmacological evaluation of sarcostemma acidium methanolic extract for anti-acneand thrombolytic activities. *Advance Pharmaceutical Journal*, 4(4):108–112. DoI: 10.31024/apj.2019.4.4.4.

Zaki AH, Saleh Gazwi HS, Hamed MM, Galal SM, Almehmadi AM, Almuraee AA, Alqurashi AF, Yassien EE. (2024) The synergistic potential of orange peel extract: A comprehensive investigation into its phenolic composition, antioxidant, antimicrobial, and functional fortification properties in yogurt. *Food Chem X. 2024* May 17; 22:101458. doi: 10.1016/j.fochx.2024.101458. PMID: 38803668; PMCID: PMC11129169.