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Analysis of Antioxidant and Antibacterial properties of *Bauhinia variegata* and *Sarcostemma acidum* through scavenging process

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ABSTRACT

The traditional use of chemical-based antimicrobial substances has adverse effects on human health. Consequently, there is a growing demand for alternative antimicrobial agents sourced from natural origins that are potentially effective and safe for human consumption. Primary aim of this research is to explore the antioxidant and antibacterial properties of leaf extracts from Bauhinia variegata and stem extract from Sarcostemma acidum, along with determining their phenolic content. The sample extracts of both the plants were evaluated for antioxidant properties using DPPH radical scavenging activity. Moreover, their antibacterial efficacy was assessed against various Gram-positive and Gram-negative bacteria at different concentrations. Both the sample extracts demonstrated significant antibacterial activity against the tested microorganisms. Additionally, the results indicated promising values for these plant extracts as natural antioxidants and antibacterials, suggesting their suitability for applications in materials requiring antioxidant and antibacterial properties.

Keywords: Antioxidant, Antibacteria, *Bauhinia variegata*, DPPH, Radical scavanging, *Sarcostemma acidum*, TPC, TFC

INTRODUCTION

Bauhinia variegata is a deciduous tree of the Leguminosae family and commonly referred to as Kachnar in Hindi and Sanskrit. It is extensively distributed across India, including the Himalayas, as noted by Sastri et al. (1950) and Ghaisas et al. (2009). Additionally, it thrives in many tropical and warm regions worldwide. This species is part of the extensive genus *Bauhinia*, comprising over 200 species of trees, shrubs, and vines renowned for their ornamental leaves and vibrant flowers. *Bauhinia variegata* typically grows as a medium-sized tree, flourishing in partial shade or full moonlight. It propagates easily through seeds and air layering. The distinctive features of this tree include deeply cordate, sub-coriaceous leaves measuring 10-15 cm in both length and breadth. Its large, fragrant flowers, either white or purplish, bloom when the tree is devoid of leaves. The hard, dehiscent pods are 15-30 cm long and 1.8-2.5 cm wide, containing 10-15 seeds, as documented in Pharmacopoeia (1989) and Kirtikar & Basu (1999). Traditionally, various parts of the plant, such as the bark, leaves, flowers, and pods, have been employed in indigenous medicinal practices due to their therapeutic properties as discussed in Kamal (2022), Bishweshwar (2023) and Sharma (2022). Pharmacological studies have investigated its potential efficacy as a remedy for diverse ailments. Likewise, *Sarcostemma acidum*, a traditional medicinal plant native to India, has garnered attention as a potential candidate for Soma plants, as indicated by Choudhary (2022) Hitesh (2023) and Vikas (2022). The Aryans utilized Soma (Somlata) to concoct 'Somras,' a rejuvenating beverage, as documented by Rajkapoor et al. (2003b) and Pandey (2018). The original source of the 'Soma' plant remains a subject of debate among Vedic and botanical scholars for over two and a half centuries. *Sarcostemma acidum* thrives in various regions of India, Pakistan, and Europe, predominantly in dry rocky areas across multiple states in India, including Bihar, Bengal, Konkan, Deccan, Tamil Nadu, Maharashtra, Madhya Pradesh, and Kerala. This plant boasts diverse medicinal properties, including bitterness, acridity, cooling effects, and rejuvenation. It has been traditionally utilized for medicinal purposes in different regions of India, with various parts of the plant used to address specific ailments. Pharmacological investigations into both *Bauhinia variegata* and *Sarcostemma acidum* have underscored their therapeutic efficacy and elucidated the mechanisms of action of their

bioactive compounds. The primary reason to explore Bauhinia variegata and Sarcostemma acidum due to their rich medicinal properties deeply rooted in traditional knowledge systems. Bauhinia variegata, known for its anti-inflammatory and anti-diabetic properties, offers potential in modern medicine. Meanwhile, Sarcostemma acidum exhibits antimicrobial and antioxidant activities, vital in combating contemporary health challenges.

(a) (b) (c) (d)

Fig. 1: Extract Preperation of plants: (a) Shaded dry leaves of *Bauhinia Variegata*, (b) Shaded dry stems of *Sarcostemma acidum*, (c) Sample of Grinded Leaves of *Bauhinia Variegata*, (d) Sample of Grinded stems of *Sarcostemma Acidum*

In today's scenario, where there's a growing need for sustainable healthcare solutions and preservation of indigenous knowledge, these plants symbolize a bridge between traditional wisdom and modern science, offering hope for holistic approaches to healthcare and environmental conservation. Considering these scenarios, many researches on medicinal properties of different plants are conducted nowadays D. Zomba (2023), R. Chuskit *et al.* (2024).

This study endeavors to elucidate the antioxidant and antibacterial properties of *Bauhinia variegata* and *Sarcostemma acidum* through DPPH Free Radical Scavenging Activity analysis. The subsequent sections outline the materials and methodology (Section II), the findings (Section III), and conclude with a summary (Section IV).

MATERIALS AND METHODS

Plant Samples & Extraction

Samples of *Bauhinia variegata* and *Sarcostemma acidum* were gathered from Shapoorji Housing Complex, Newtown, Kolkata, India (coordinates 22.5754N; 88.4798 E). These specimens underwent identification by an expert at the Center for Microbiology and Bio-Technology (CMBT), a research and training institute situated in Bhopal, M.P., India. Fig. 1 (a) and (b) depict the shaded dry leaves and stems' samples of *Bauhinia variegata* and *Sarcostemma acidum* respectively. In Fig. 1 (c) and (d), the samples were manually ground. Subsequently, to acquire the extracts, the machine-ground leaves and stems (250 g) underwent extraction using ethanol solvent at room temperature (25˚C ± 2˚C). The resultant extract was concentrated by evaporating the solvent under reduced pressure at 40˚C using a rotary evaporator, resulting in a gummy concentrate with a dark green hue.

Total Flavonoid Content (TFC) and Total Phenolic Content Estimation

The determination of flavonoid content in the isolated crude extracts of *Bauhinia variegata* leaves and *Sarcostemma acidum* stem was conducted following the procedure outlined by Jia et al. (1999), where a 0.5 ml of sample of each plant extract and 1.25 ml of distilled water is kept in a clean separate test tubes. Then each sample is examined under the different quantities of Na_2O_3, Al_2O_3, NaOH, solution for specific time intervals and absorbance of the mixture is measured at 510 nm.

Similarly, the total phenolic content of the isolated crude extracts from *Bauhinia variegata* leaves and *Sarcostemma acidum s*tem was determined following the protocol outlined by Singleton & Rossi (1965). Here, the samples

were analyzed under Folin and Ciocalteu's phenol reagent. After the Na_2O_3 mixture the sample were kept in dark and examined through UV-Vis spectrophotometer at an absorbance of 760 nm. Both the flavonoid and Phenolic contents are expressed as mg quercetin equivalents per gram of sample and mg of tannic acid equivalents per gram of sample respectively.

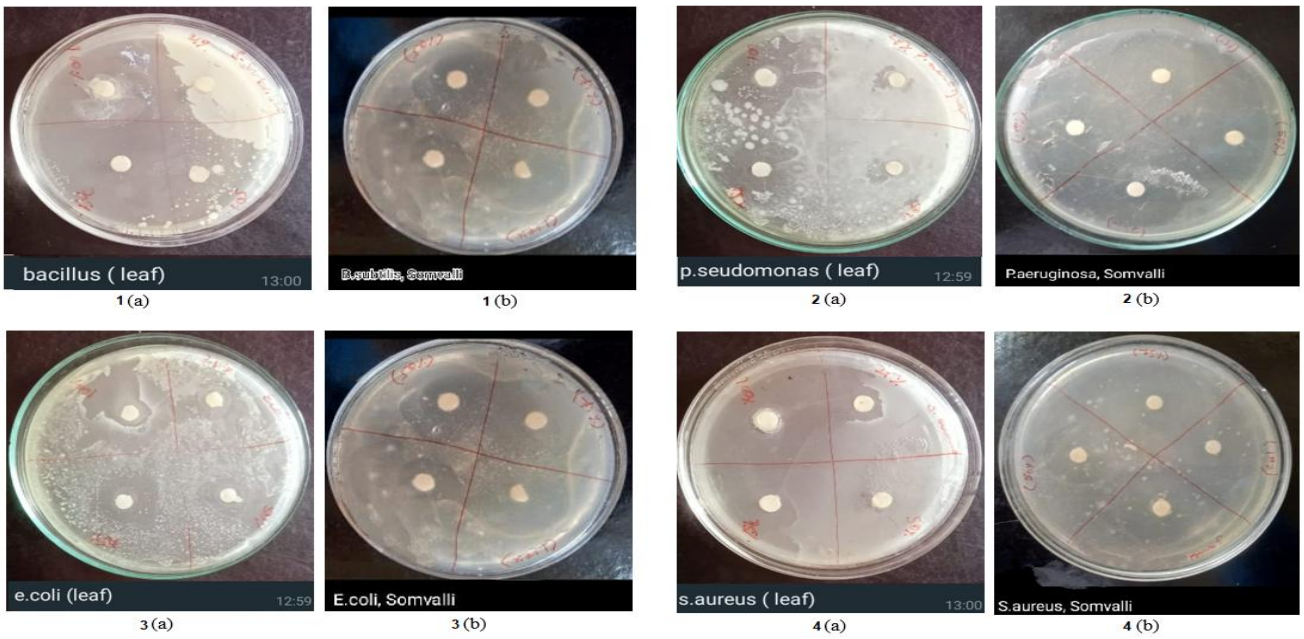
DPPH Assay

The capacity of these compounds to counteract the stable radical DPPH was assessed to determine their efficacy in scavenging free radicals. To investigate the antioxidant potential through the DPPH radial scavenging method, the procedure outlined in Benzie & Strain (1996) is followed. Extracts of the samples and standards ascorbic acid were formulated to 5 different concentrations ranging from 20 to 100 ppm. After following the aforementioned method and incubation, the percentage inhibition for both the samples were determined at 517 nm against ethanol using a UV-Vis Spectrophotometer.

$$\% \text{ Inhibition} = \left[1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Control}}\right] \times 100$$

Antibacterial Activity Analysis

Different microbial samples from various oral flora were identified using the swab method to measure antibacterial activity. These samples were then spread on specific media plates and incubated at 37˚C temperature. Following the incubation, the bacterial isolates were subjected to gram staining and test is conducted using a compound microscope at 100 times enlargement. Based on morphometric characterization, four distinct bacterial isolates were identified as E.coli, P.aeruginosa, B.subtilis, and S.aureus, belonging to both gram-positive (GP) and gram-negative (GN) categories. To test the antibacterial properties of the extractives against these four bacterial species, the researchers employed the disc diffusion method, as mentioned in Bauer et al. (1966). As mentioned in the disc diffusion method the ample disc was prepared as shown in the Fig. 2. The lack of development surrounding the disc is referred as “Zone of Inhibition”.



1 (a) 1 (b) 2 (a) 2 (b)

3 (a) 3 (b) 4 (a) 4 (b)

Fig. 2: Antimicrobial Activities of *Bauhinia variegata* leaves abstract; and *Sarcostemma acidum* stem abstract respectively against; 1(a) and 1(b) Bacillus Cereus; 2(a) and 2(b) P. Seudomonas; 3(a) and 3(b) E.Coli; 4(a) and 4(b) S.Aures

RESULTS & DISCUSSION

Basic Phytochemical Analysis

Table 1: Phytochemical Analysis of the extract of *Bauhinia variegata* (*Bauhinia variegata*) and *Sarcostemma acidum* (*Sarcostemma acidum)*

Extract	Tannin	Flavonoid
Ethenolic Extract of *Bauhinia variegata* leaves	Present	Present
Ethenolic Extract of *Sarcostemma* acidum stems	Present	Present

The samples underwent qualitative testing to identify its chemical constituents such as flavonoids and tannins. These tests followed the methodology outlined in Dev et al. (2017), and a 10% (w/v) solution of the samples were used in every single of the conducted test until it is not otherwise specified. The initial screening of the leaf extract from *Bauhinia variegata* plant and the stem extract from *Sarcostemma acidum* plant using different solvents displayed the occurrence of the bioactive components like flavonoids and tannins. A list of the results from the phytochemical tests can be found in Table 1.

Determination of TPC and TFC

To determine the TPC in the extracts, the F-C assay was utilized, employing Tannic acid as a standard reference compound, as illustrated in Fig. 3. The F-C assay is a rapid and straightforward method aiding in the characterization and standardization of botanical samples. This technique relies on the oxidation of phenolics by molybdotungstate present in the F-C reagent, resulting in the formation of a colored product with a maximum absorption wavelength (λ_{max}) of 765 nm, as described by Prior et al. (2005). The TPC of the plant extracts, determined using F-C's reagent, is presented in tannic acid equivalent, employing the following standard curve equation: y = 0.0061x + 0.7264, with an R^2 value of 0.9975. The TPC in the ethanol extracts of *Bauhinia variegata* leaves and *Sarcostemma acidum* stems is quantified as 17.48 mg TA/g and 18.83 mg TA/g, respectively.

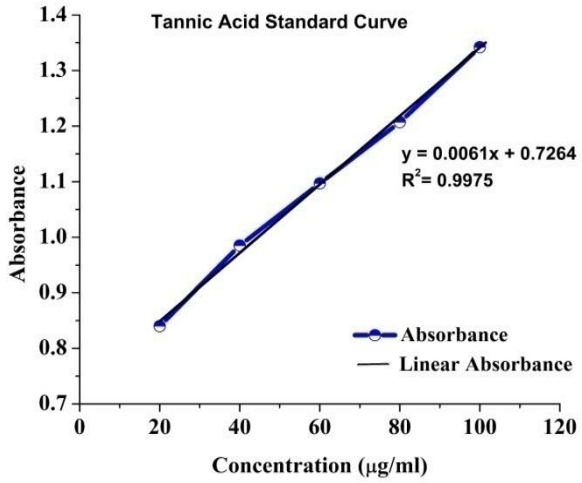


Fig. 3: Calibration graph of Tannin Acid

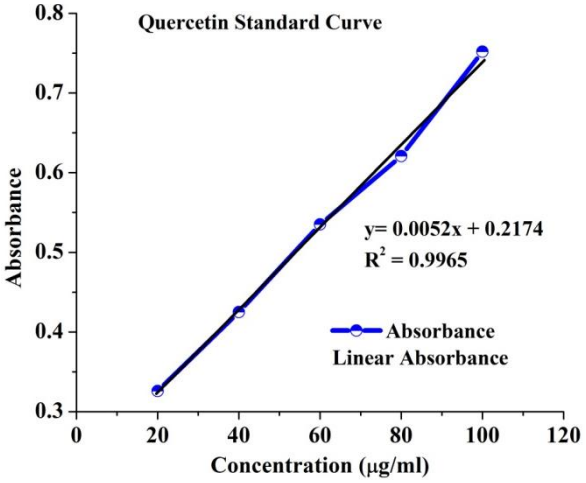


Fig. 4: Calibration graph of Qurecetin

Similarly, the quantification of flavonoids in *Bauhinia variegata* leaves and *Sarcostemma acidum* stems was conducted utilizing the spectrophotometric method employing aluminum chloride. The TFC of the samples was assessed by comparison with quercetin, employed as the standard compound, as illustrated in Fig. 4. The flavonoid content was expressed as quercetin equivalent, utilizing the standard curve equation: y = 0.0052x + 0.2174, with an R^2 value of 0.9965, measured in mg of quercetin per gm of sample. The *Bauhinia variegata* leaves extracts exhibited a flavonoid concentration of 17.49 mg/g, whereas the *Sarcostemma acidum* stems extract showed a concentration of 19.48 mg/g.

DPPH Radical Scavenging

As per the DPPH method, the ethanol extract of *Bauhinia variegata* leaves displayed scavenging activity against free radicals at different concentrations. The scavenging percentages at 20, 40, 60, 80, and 100 mg/ml were determined to be 8.84%, 28.1%, 41.17%, 57.68%, and 82.45%, respectively (as illustrated

in Fig. 5). Similarly, the extract from *Sarcostemma acidum* stems exhibited DPPH radical inhibition at 23.12%, 42.7%, 68.53%, 75.72%, and 86.53% at the same concentrations

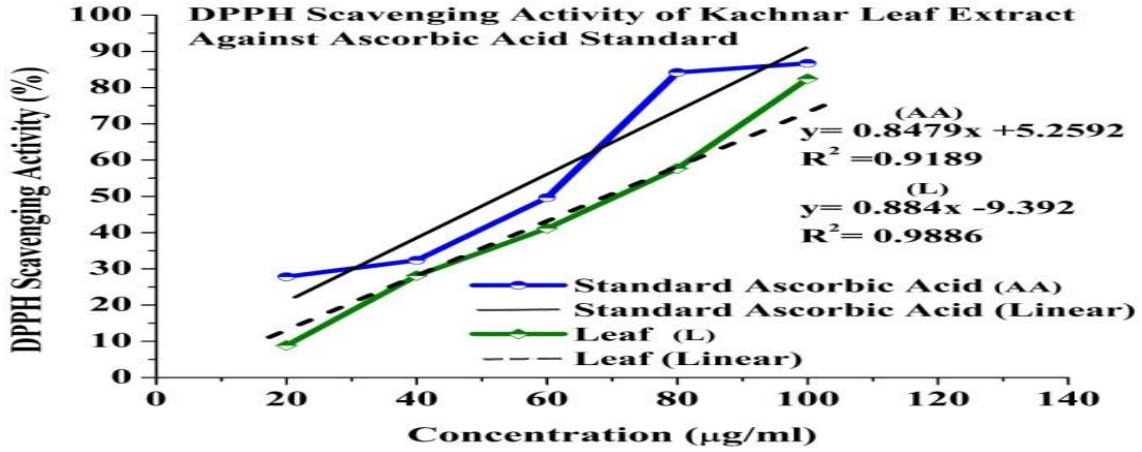


Fig. 5: DPPH Scavenging Activity of *Bauhinia variegata* Leaf Extract against Acid Standard Ascorbic

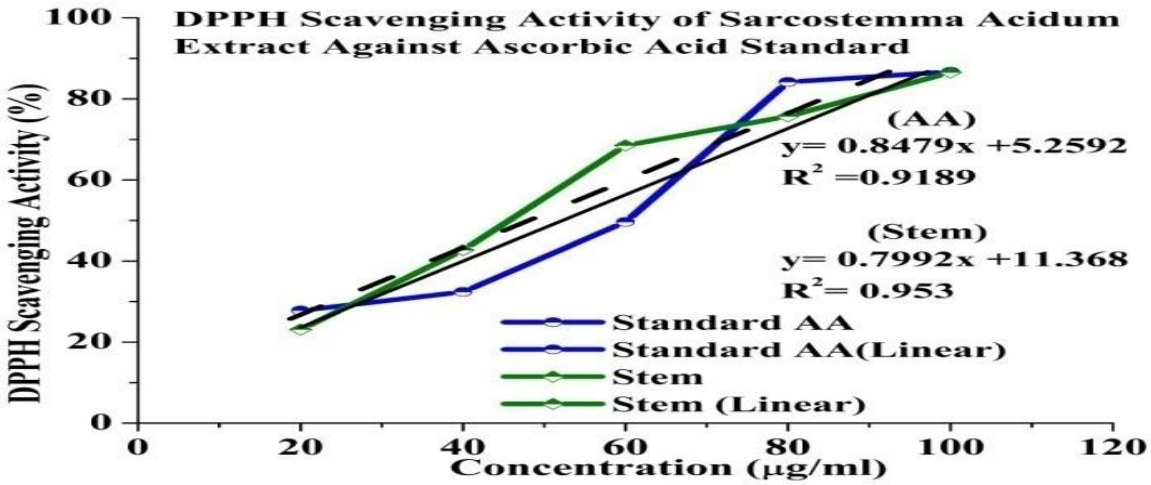


Fig. 6: DPPH Scavenging Activity of *Sarcostemma acidum* Stem Extract against Ascorbic Acid Standard

(as depicted in Fig. 6). Furthermore, the DPPH assay indicated that the IC_{50} values for ascorbic acid, *Bauhinia variegata* leaf extract, and *Sarcostemma acidum* stem extract were 0.18, 0.39,and 0.81(all in mg/ml) respectively. These findings suggest that the extracts from *Bauhinia variegata* leaves and *Sarcostemma acidum* stems possess the capability to scavenge diverse free radicals across various systems. Hence, they hold promise as valuable beneficial elements for mitigating radical-induced hazards.

Antibacterial Activity Analysis

In this analysis, two gram positive namely B. subtilis, and S. aureus, and two gram negative E. coli, P. aeruginosa have been chosen for analysis of antibacterial characteristics analysis of both the samples. All examined samples shows antibacterial activity at a concentration of 20% (w/v). Consequently, this concentration was further employed to determine their minimum inhibitory concentrations (MIC) utilizing the agar well diffusion method. Additionally, it was utilized to evaluate their efficacy in controlling foodborne pathogens and spoilage microorganisms, as described by Ashraf et al. (2018). The assessment of antibacterial activity for both plant extracts is depicted in Fig. 7 and Fig. 8, respectively. The results indicated that both these plants samples showed potential effectiveness in inhibiting microbial growth, albeit with varying degrees of potency. Among the tested pathogenic bacteria, the *Bauhinia variegata* leaf extract exhibited the highest efficacy in inhibiting the growth of S. aureus at a concentration of 25 mg/ml.

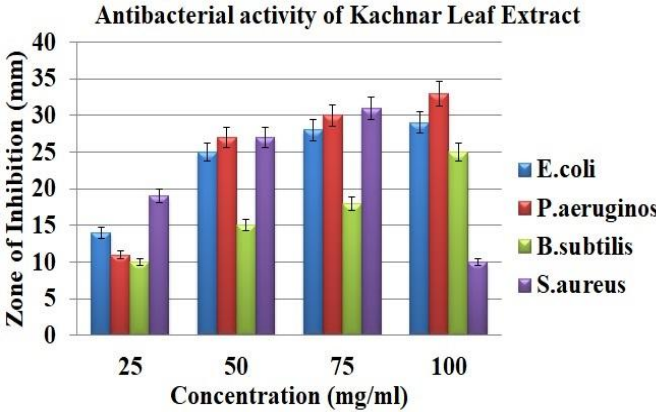


Fig. 7: Antibacterial activity of *Bauhinia Variegata* Leaf Extract

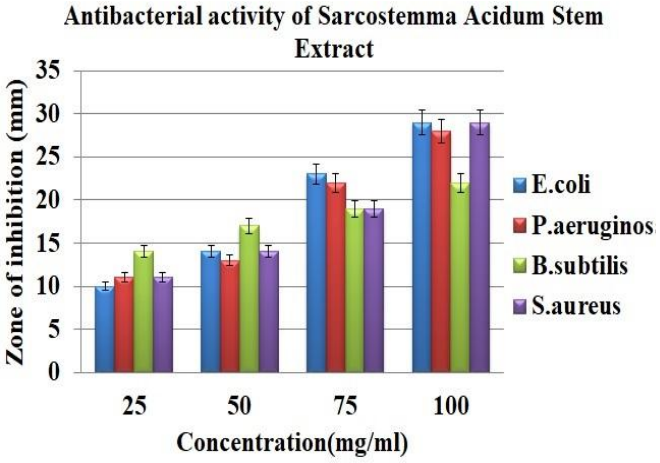


Fig. 8: Antibacterial activity of *Sarcostemma Acidum* Stem Extract

Conversely, the *Sarcostemma acidum* stem extract displayed the greatest effectiveness against B. subtilis at the same concentration. However, at a concentration of 50 mg/ml, the *Bauhinia variegata* leaf extract demonstrated the most significant inhibition of E. coli, P. aeruginosa, and S. aureus growth among the tested pathogenic bacteria, while the *Sarcostemma acidum* stem extract exhibited enhanced performance specifically against E. coli. At a concentration of 75 mg/ml, the *Bauhinia variegata* leaf extract showed moderate effectiveness in inhibiting B. subtilis growth among the tested pathogenic bacteria, while the *Sarcostemma acidum* stem extract exhibited considerable efficacy against all microorganisms at the same concentration. Finally, at a concentration of 100 mg/ml, the *Bauhinia variegata* leaf extract displayed relatively less effectiveness in retarding S. aureus growth among the tested pathogenic bacteria, whereas the *Sarcostemma acidum* stem extract demonstrated comparable effectiveness against all microorganisms at the same concentration.

CONCLUSION

In conclusion, the study results underscore the promising potential of *Bauhinia variegata* and *Sarcostemma acidum* extracts as natural alternatives to chemical preservatives. This research successfully conducted and demonstrated DPPH scavenging activity, TPC and TFC analysis, and antibacterial activity. Both the leaf extract of *Bauhinia variegata* and the stem extract of *Sarcostemma acidum* exhibit significant antioxidant and antibacterial properties, advocating these plants possible uses as natural drugs, preservatives, and in various other suitable applications.

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