

## Anti-infective Properties of *Brachychiton rupestris* and *Brachychiton luridum* Leaves and their Qualitative Phytochemical Screening

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### Abstract

*Brachychiton rupestris* and *Brachychiton luridum* are deciduous trees belonging to family Malvaceae. Several members of the genus *Brachychiton* and its related genus, *Sterculia*, are used in folk medicine for the treatment of infections. Herein, we investigated the antimicrobial activity of the total extracts, *n*-hexane, dichloromethane as well as the ethyl acetate fractions of the leaves of both species using agar well diffusion method in an effort to better consolidate the scientific basis for their traditional uses. The study was carried out against two Gram-positive and two Gram-negative bacteria together with two fungi. The extracts and fractions showed mean inhibition zones diameter in the range of 10.97-14.98 mm with minimum inhibitory concentration values ranging between 39.06 and 625 µg/mL against the susceptible bacteria and fungi. The ethyl acetate fraction of *B. luridum* exhibited notable activity against *Staphylococcus aureus* while the total extract showed moderate activity against *Salmonella typhi*. Regarding *B. rupestris*, the total extract, *n*-hexane and ethyl acetate fractions showed moderate activity against *Escherichia coli*, *Candida albicans*, and *Salmonella typhi*, respectively. Other extracts and fractions showed weak or no activity against the tested microorganisms. Qualitative phytochemical screening of both plants was performed to preliminary unveil the classes of secondary metabolites present in the leaves. The results indicated the existence of flavonoids, tannins, sterols, triterpenes, carbohydrates and/or glycosides in both plants.

**Keywords:** Anti-infective activity; *Brachychiton luridum*; *Brachychiton rupestris*; Malvaceae; Phytochemical screening

### Abbreviations

AIDS: Acquired immune deficiency syndrome; B: *Brachychiton*; BLDCM: Dichloromethane fraction of *B. luridum* leaves; BLEt: Ethyl acetate fraction of *B. luridum* leaves; BLHex: Hexane fraction of *B. luridum* leaves; BLT: Total extract of *B. luridum* leaves; BRDCM: Dichloromethane fraction of *B. rupestris* leaves; BREt: Ethyl acetate fraction of *B. rupestris* leaves; BRHex: Hexane fraction of *B. rupestris* leaves; BLT: Total extract of *B. luridum* leaves; BRT: Total extract of *B. rupestris* leaves; CFU: Colony forming unit; DCM: Dichloromethane; DMSO: Dimethyl sulfoxide; HIV: Human immunodeficiency virus cancer; MIC: Minimum inhibitory concentration; NA: No activity; NT: Not tested; RCMB: Regional center for mycology and biotechnology; S: *Sterculia*; SD: Standard deviation.

### Introduction

The last century witnessed a great renaissance in the interest and use of medicinal plants. Researchers established scientific protocols for studying the biological activities of medicinal plants. It was found that the therapeutic effects of plants are mainly attributed to the existence of various classes of secondary metabolites that are endogenously produced in terrestrial plants. Scientific studies revealed a myriad of therapeutic activities for plants extracts and secondary metabolites exemplified by cytotoxic, antimicrobial, anti-inflammatory, analgesics, and antidepressant activities. Noteworthy to mention that these

phytochemicals demonstrate important biological activities to the plant itself in the form of protection against predators such as insects, fungi, bacteria, and viruses [1]. Such activities suggested the potential use of plants extracts and their secondary metabolites against human and animal pathogens.

Infectious diseases represent one of the major causes of morbidity and mortality worldwide [2]. Nowadays, the high prevalence of infectious diseases and the lack of new antibiotics in the drug pipeline causes alert on the global level. Undoubtedly the uncontrolled use of antibiotics on regular basis resulted in the development of bacterial resistant that represent a critical health problem [3-5]. World Health Organizations and other health regulatory authorities issued alarming reports on the future of humanity in post antibiotic era. They encourage governmental bodies and private companies to join forces and focus on developing new antimicrobial agents. Medicinal plants continue to be a rich source for the discovery of new molecular entities with potential antimicrobial activity [3,4].

Malvaceae is a family of flowering plants with more than 200 genera and nearly 2300 species [6,7]. The family is composed mainly of tropical trees and shrubs with a few climbing and herbaceous species showing a wide array of medicinal values [6-8]. *Brachychiton* is a small genus belonging to family Malvaceae with approximately 30 species; native to Australia [9]. Most of the species in *Brachychiton* are monoecious trees or shrubs, deciduous or evergreen, with cylindrical or swollen trunks, so it is known as the "bottle tree". Previously, members of the genus were included under *Sterculia* but after the thorough examination of the morphology of the species, they were retained as a separate genus from *Sterculia* [9].

Phytochemical investigation of *Brachychiton* sp. led to the isolation of different classes of compounds such as sterols, triterpenes, flavonoids, phenolic acids, coumarins, and alkaloids [10-13]. Fewer reports studied the biological activities of plants belonging to this genus and it was found that *Brachychiton* sp. exhibited antibacterial, antioxidant [14], *antischistosomal* [15] and antidiabetic activities [11,13].

*B. rupestris* is native to Queensland and it is known as Queensland bottle tree, while *B. luridum* (syn. *B. discolor*) is commonly known as Lacebark tree [16]. The methanol extract of *B. rupestris* leaves was previously investigated for its effectiveness against *Schistosoma mansoni* [15]. Also, the isolated mucilage from the leaves demonstrated a significant hypoglycemic effect in experimental animals [17]. From the leaves of *B. rupestris* different flavonoid aglycones and glycosides were isolated [17]. Numerous classes of compounds were isolated from *B. luridum* leaves as sterols, triterpenes flavonoid aglycones and glycosides, whereas alkaloids were isolated from the seeds [11].

The aim of the present study was to assess the antimicrobial activity of *B. rupestris* and *B. luridum* leaves extract and fractions. The absence of any report regarding the antimicrobial activity of both species along with the interesting folk medicinal uses of the related genus, *Sterculia*, acted as the driving force to accomplish this study. Qualitative phytochemical screening of both plants was performed to identify major classes of phytoconstituents that could be responsible for the observed activity.

## Materials and Methods

### Plant material

Samples of *B. rupestris* leaves (T. Mitch. ex Lindl) K. Schum and *B. Luridum* C. Moore were collected from Orman botanical garden, Giza, Egypt, in June, August and September 2014. The plants were kindly authenticated morphologically by Dr. Mohamed El-Gibaly, Department of Botany, National Research Centre (NRC), Giza, Egypt. Voucher specimens with codes PHG-P-BR-198 and PHG-P-BL-199 for *B. rupestris* and *B. luridum*, respectively, are retained at the herbarium of Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

### Extraction and fractionation

The crushed air-dried leaves of *B. rupestris* (3.05 kg) were percolated in distilled methanol (29 L × 3) then filtered. The filtrate was completely evaporated under vacuum at low temperature (45°C) till dryness to give 333.56 g dried total methanol extract. The dried extract (300 g) was then fractionated with n-hexane, dichloromethane, ethyl acetate and butanol, successively to give the corresponding fractions with the following weights 54.35, 8.54, 5.91 and 9.1 g, respectively. The remaining hydromethanolic extract was 191.5 g.

For *B. luridum*, the leaves (600 g) were percolated in (6 L × 3) distilled methanol then filtered. The total methanol extract was 36 g after complete evaporation under reduced pressure at low temperature (45°C). A portion of the solid residue (11 g) was then fractionated as previously mentioned to give n-hexane, dichloromethane, and ethyl acetate fractions, with the following weights 1.3, 1.2, and 0.9 g, respectively. The remaining hydromethanolic extract was 6.4 g.

## Evaluation of antimicrobial activity

**Bacterial and fungal strains:** Antimicrobial susceptibility test was done at the Regional Center for Mycology and Biotechnology (RCMB) at the Antimicrobial Unit, Al-Azhar University, Cairo, Egypt. The susceptibility tests were performed according to NCCLS recommendations (National Committee for Clinical Laboratory Standards, 1993). The antimicrobial activity of the samples was determined using agar well diffusion method [18]. All samples were tested *in vitro* for their antibacterial activity against two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*, and two Gram-negative bacteria, *Salmonella typhi* and *Escherichia coli* using nutrient agar medium. Antifungal activity was carried out against *Aspergillus flavus* and *Candida albicans* using malt agar medium. Gentamycin was used as the standard drug for Gram-positive and Gram-negative bacteria while ketoconazole was used for fungi. All antibiotics were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). DMSO was used as the negative control. The samples were tested at a concentration of 5 mg/mL against both bacterial and fungal strains.

### Determination of the mean zones of inhibition

Screening tests regarding the inhibition zone were carried out by the well diffusion method [18]. The inoculum suspension was prepared from colonies grown overnight on an agar plate and inoculated into Mueller-Hinton broth (fungi using malt broth). A sterile swab was immersed in the suspension and used to inoculate Mueller-Hinton agar plates (fungi using malt agar plates) and to consistently distribute microbial suspension over the surface of solidified media. The extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration (5 mg/mL) and solutions of the tested samples were added to each well with the help of micropipette, the inhibition zone was measured in mm scale around each well after 24 h at 37 °C. Controls using DMSO were adequately tested.

### Determination of the Minimum Inhibitory Concentration (MIC)

The MICs were determined using agar plate method following the method of Hindler et al. Nutrient agar (for bacteria) and malt agar (for fungi). The media were sterilized in an autoclave for 25 min at 121°C and allowed to cool to 45°C. Two-fold serial dilutions of each sample were added to the medium immediately then it was poured into the Petri dishes. DMSO was used as the negative control. The culture of each organism in the nutrient medium was diluted with sterile distilled water to 105-106 CFU/mL. A loop of each suspension was inoculated in the appropriate medium with the sample or the control added. Then the plates were incubated at 37°C for 24 hours for bacteria, and at 28°C for three to four days for fungi. The MIC was the lowest concentration that completely inhibited the visible growth of microorganisms compared with the control [19].

### Qualitative phytochemical screening of *B. rupestris* and *B. luridum* leaves

**The powdered dried sample of *B. rupestris* leaves was screened for the following constituents:** flavonoids, sterols and/or triterpenes, carbohydrates and/or glycosides, saponins, tannins, alkaloids, and anthraquinones following methods reported in previous literature [20,21]. The color intensity or precipitate formation was used as analytical responses to these tests.

### Test for flavonoids

**Schinoda's test:** The methanol extract (5 mL) was added to concentrated hydrochloric acid (0.5 mL) followed by few mg of magnesium turnings. A pink or red coloration indicates the presence of flavonoids [22].

### Tests for steroids and/or triterpenoids

**Liebermann-Burchard's test:** 0.3 mL of acetic anhydride was added to 1 mL chloroform solution of the extract, and few drops of conc. sulfuric acid were added along the side of the tube. A reddish violet color at the junction of the two layers shows the presence of sterols and/or triterpenes [20].

**Salkowski's test:** 1 mL of conc. sulfuric acid was added on the side of a tube containing a chloroformic solution of the extract. Sterols give reddish brown coloration of the interface [23].

### Tests for carbohydrates and/or glycosides

**Molisch's test:** The aqueous extract (2 mL) of was mixed with 0.2 mL of ethanolic  $\alpha$ -naphthol (20%) and 2 mL of sulfuric acid along test tube side to form two layers. A violet ring at the junction between the two layers indicates a positive result for carbohydrates [24].

**Fehling's test (for reducing sugars):** Solution A: 34.64 g of copper (II) sulfate were dissolved in a mixture of 0.5 mL sulfuric acid and sufficient amount of water to produce 500 mL solution.

Solution B: 176 g of sodium potassium tartrate and 77 g of sodium hydroxide were dissolved in sufficient amount of water to produce 500 mL solution. The aqueous extract was mixed with equal volumes of solutions A and B and left in a water bath. A red precipitate is specific for reducing sugar(s) [25].

### Test for saponins

**Froth test:** 1 g of the plant sample was boiled in 10 mL water for a few minutes; filtered and few volumes of distilled water were added to 1 mL of the filtrate in a test tube. The solution was shaken vigorously and observed for a stable persistent froth which indicates that saponins are present [26].

### Test for tannins

**Ferric chloride test:** 1 g of  $\text{FeCl}_3$  was dissolved in water and completed to 100 mL. 2 mL of the aqueous extract was added to 2 mL of water then 1 mL of diluted ferric chloride solution was added. Condensed tannins give a green color while hydrolyzable tannins give a blue-black color [21,26].

### Test for alkaloids

**Mayer's reagent:** 5 g of potassium iodide was dissolved in 20 mL water then 1.36 g of mercuric chloride in 60 mL water was added and

completed with water to 100 mL solution. 3 drops of this reagent were added to an alcoholic extract and dissolved in 1 mL dil. HCl. The formation of creamy white precipitate or turbidity is positive for alkaloids [27].

### Tests for anthraquinones

**Bortrager's test (for anthraquinone aglycone):** The powdered material was macerated in benzene then filtered. Aqueous ammonia was added to the filtrate and shaken. A rose pink to red color of ammonia layer shows that anthraquinone aglycones exist [28].

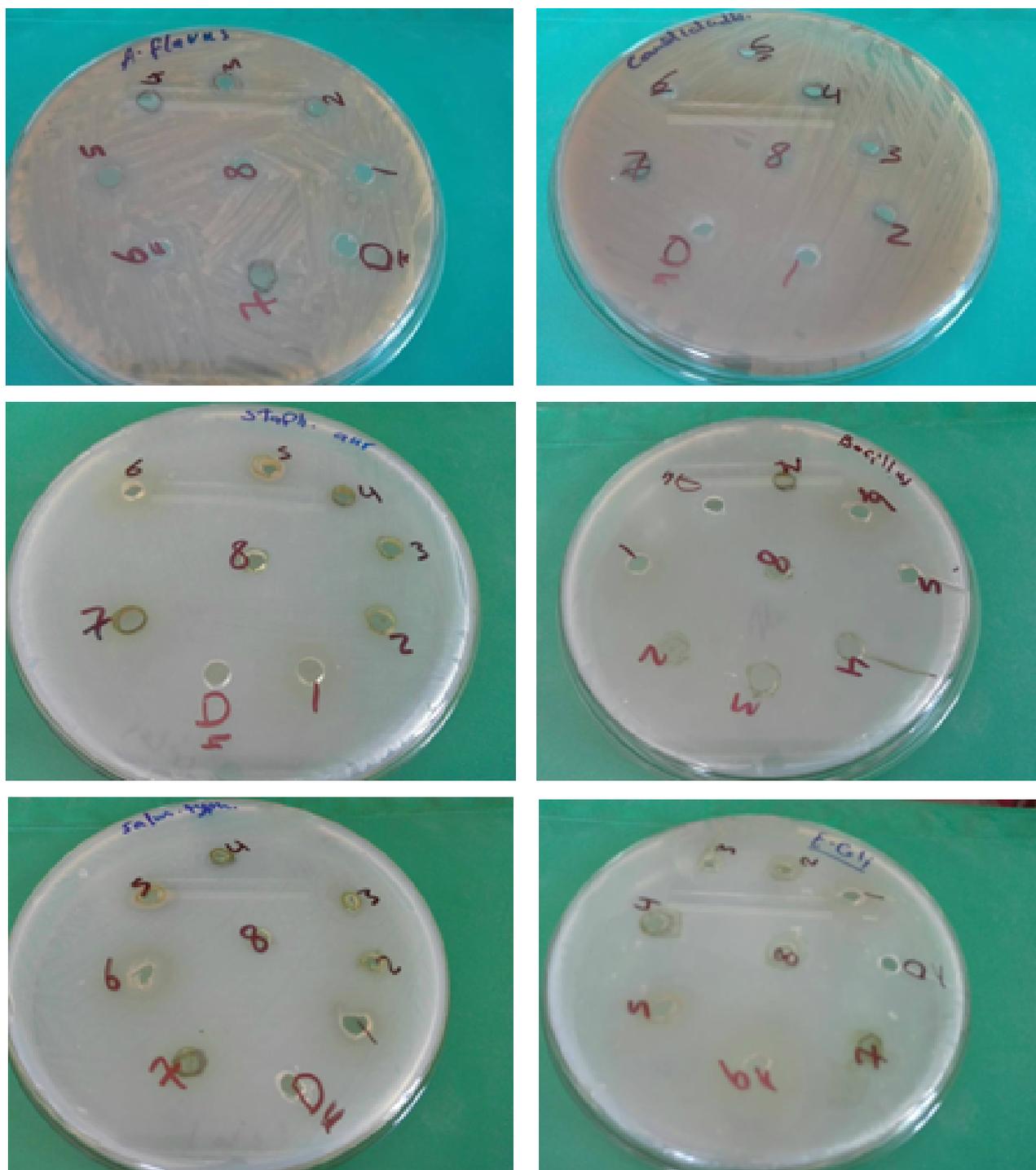
**Modified Bortrager's test (for anthraquinone glycosides):** The powdered material was first hydrolyzed with alcoholic potassium hydroxide and then treated as in Bortrager's test. A rose pink to red color of the ammoniacal layer gives an indication of anthraquinone glycoside [28].

## Results

### Antimicrobial activity of *B. rupestris* and *B. luridum* leaves extracts and fractions

The antimicrobial activity of *B. rupestris* and *B. luridum* leaves extracts and fractions was assessed by the agar well diffusion method at 5 mg/mL and MICs were determined (Tables 1 and 2) (Figure 1). In general, the extracts and fractions showed MIC values between 39.06-5000  $\mu\text{g/mL}$  for the susceptible bacteria and fungi. It was reported that MIC values below 100  $\mu\text{g/mL}$  indicate good activity; whereas values from 100 to 500  $\mu\text{g/mL}$  are considered moderate; while those from 500 to 1000  $\mu\text{g/mL}$  are weak; and over 1000  $\mu\text{g/mL}$  are inactive [29].

The results showed that the ethyl acetate fraction of *B. luridum* showed notable activity against *Staphylococcus aureus* where MIC value was 39.06  $\mu\text{g/mL}$  and the mean inhibition zones diameter was 11.98 mm. The total extract of *B. luridum* and the ethyl acetate fraction of *B. rupestris* displayed moderate activity against *Salmonella typhi* with mean zones of inhibition of 10.97 mm and 11.81 mm, and MIC values of 156.32  $\mu\text{g/mL}$  and 156.23  $\mu\text{g/mL}$ , respectively. The total extract of *B. rupestris* showed also moderate activity against *Escherichia coli* with mean zones of inhibition diameter at 12.98 mm and MIC value of 312.5  $\mu\text{g/mL}$ . The hexane fraction of *B. rupestris* exhibited moderate activity against *Candida albicans* showing the mean inhibition zones diameter of 12.98 mm and MIC value of 312.5  $\mu\text{g/mL}$ . However, the dichloromethane fraction of *B. luridum* showed weak activity against *Escherichia coli* with MIC at 625  $\mu\text{g/mL}$  and mean inhibition zone of 14.98 mm. Other extracts and fractions showed very weak or no activity against the tested microorganisms with MIC values exceeding 1000  $\mu\text{g/mL}$ . The total extract and ethyl acetate fraction of *B. rupestris* exhibited very weak activity against *Candida albicans* and *Escherichia coli*, respectively. Also, both the dichloromethane fraction of *B. rupestris* and the total extract of *B. luridum* showed very weak activity against *Staphylococcus aureus*.



**Figure 1:** Mean inhibition zones for *B. rupestris* and *B. luridum* leaves extracts and fractions using agar well diffusion method against different pathogenic bacteria and fungi. A: *Aspergillus flavus*, B: *Candida albicans*, C: *Staphylococcus aureus*, D: *Bacillus subtilis*, E: *Salmonella typhi*, F: *Escherichia coli*, 1: Ethyl acetate fraction of *B. luridum*, 2: dichloromethane fraction of *B. rupestris*, 3: total extract of *B. luridum*, 4: hexane fraction of *B. rupestris*, 5: total extract of *B. rupestris*, 6: ethyl acetate fraction of *B. rupestris*, 7: dichloromethane fraction of *B. luridum*, 8: hexane fraction of *B. luridum*

Microorganisms		Diameter of inhibition zone (mm)								
		BRTa	BRHexb	BRDCM <sub>c</sub>	BREtd	BLTe	BLHexf	BLDCMg	BLEth	Control
Fungi										Ketoconazole
	<i>Aspergillus flavus</i> (RCMB 002002)	NA	NA	NA	NA	NA	NA	NA	NA	16 ± 0.02
	<i>Candida albicans</i> (RCMB 005003 ATCC 10231)	11 ± 0.02	12.98 ± 0.02	NA	NA	NA	NA	NA	NA	23.03 ± 0.04
Gram-Positive										Gentamycin
	<i>Staphylococcus aureus</i> (RCMB 010010)	NA	NA	11.03 ± 0.06	NA	11.99 ± 0.01	NA	NA	11.98 ± 0.13	30.01 ± 0.01
	<i>Bacillus subtilis</i> (RCMB 015(1) NRRL B-543)	NA	NA	NA	NA	NA	NA	NA	NA	26.02 ± 0.03
Gram-Negative										Gentamycin
	<i>Salmonella typhi</i> (RCMB 006 (1) ATCC 14028)	NA	NA	NA	11.81 ± 0.07	10.97 ± 0.06	NA	NA	NA	33.02 ± 0.03
	<i>Escherichia coli</i> (RCMB 010052 ATCC 25955)	12.98 ± 0.08	NA	NA	11.98 ± 0.13	NA	NA	14.98 ± 0.08	NA	27.09 ± 0.01

**Table 1:** Mean inhibition zones in mm beyond well diameter (6 mm) produced on a range of pathogenic microorganisms. a: Total extract of *B. rupestris*, b: hexane fraction of *B. rupestris*, c: dichloromethane fraction of *B. rupestris*, d: ethyl acetate fraction of *B. rupestris*, e: total extract of *B. luridum*, f: hexane fraction of *B. luridum*, g: dichloromethane fraction of *B. luridum*, h: ethyl acetate fraction of *B. luridum*. Data are measured in triplicates (n=3) and presented as means ± S.D, well diameter: 6.0 mm (100 µl were tested). Concentration of tested samples=5 mg/mL. NA: No activity.

Microorganisms		Minimum Inhibitory Concentration (µg/mL)								
		BRT	BRHex	BRDCM	BREt	BLT	BLHex	BLDCM	BLEt	Control
Fungi										Ketoconazole
	<i>Aspergillus flavus</i> (RCMB 002002)	NT	NT	NT	NT	NT	NT	NT	NT	
	<i>Candida albicans</i> (RCMB 005003 ATCC 10231)	5000	312.5	NT	NT	NT	NT	NT	NT	
Gram-Positive										Gentamycin
	<i>Staphylococcus aureus</i> (RCMB 010010)	NT	NT	1250	NT	1250	NT	NT	39.06	
	<i>Bacillus subtilis</i> (RCMB 015(1) NRRL B-543)	NT	NT	NT	NT	NT	NT	NT	NT	
Gram-Negative										Gentamycin
	<i>Salmonella typhi</i> (RCMB 006 (1) ATCC 14028)	NT	NT	NT	156.23	156.32	NT	NT	NT	
	<i>Escherichia coli</i> (RCMB 010052 ATCC 25955)	312.5	NT	NT	1250	NT	NT	625	NT	

**Table 2:** Minimum Inhibitory Concentrations (MIC) in µg/mL for the tested microorganisms. NT: Not tested. RCMB: The regional center of mycology and biotechnology, Al-Azhar University

### Qualitative phytochemical screening of *B. rupestris* and *B. luridum* leaves

The results illustrated in Table 3, revealed the presence of flavonoids, sterols and/or triterpenes, carbohydrates and/or glycosides,

tannins in both *B. rupestris* and *B. luridum* leaves. No anthraquinones, alkaloids, and saponins were detected. These results were in agreement with the previously reported data about the compounds isolated from both species [11,17].

Phytoconstituents	<i>B. rupestris</i>	<i>B. luridum</i>
Flavonoids	+	+
Sterols and / or triterpenes	+	+
Carbohydrates and/or glycosides	+	+
Saponins	-	-
Tannins	+	+
Alkaloids	-	-
Anthraquinones	-	-

**Table 3:** Results of phytochemical screening of *B. rupestris* and *B. luridum* leaves. (+) present, (-) absent.

## Discussion

Since antiquity, man has used medicinal plants to treat common infectious diseases [30]. Different members of *Sterculia* and its most closely related genus, *Brachychiton*, proved effective in eradicating bacterial infections leading to diarrhea and dysentery [31]. Leaves of *S. africana* are used in Tanzania by traditional healers to treat fungal infections [32]. *S. lychnophora* dried ripe seeds are also used to cure sore throat and pharyngitis [33,34]. A decoction of the powdered dried leaves of *S. setigera* has been used for the treatment of mycobacterium especially in HIV/AIDS patients with chronic cough and blood stains. The root bark and fruits of *S. setigera* are used to treat dysentery, syphilis, boils, and inflammation [35]. The leaves of *S. striata* together with hot butter or olive oil are used against boils and furuncles [7,36].

Numerous members of *Brachychiton* and *Sterculia* showed antimicrobial activity against different microorganisms. The methanol and ethyl acetate extracts of the wood branches of *B. diversifolius* exhibited potent antibacterial activity against Gram-negative bacteria including *Escherichia coli*, *Salmonella typhi*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*, the activity was lower against Gram-positive bacteria [37]. *S. foetida* leaves ethanol and bark methanol extracts showed antimicrobial activity against *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* [38,39]. *S. setigera* acetone, hexane and dichloromethane extracts of the leaves, also petroleum ether, acetone and methanol extracts of the stem bark showed antibacterial against *Klebsiella pneumonia*, *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus cereus*, and a virulent strain of *Mycobacterium tuberculosis* (H37RV) [35,40,41]. Also, *S. villosa* leaves ethanol and bark methanol extracts showed activity against the growth of *Salmonella paratyphi* and *Pityrosporum ovale* [42]. Wilson et al. found that the methanol extract of *S. quinqueloba* leaves exhibited potent activity against different pathogenic bacteria and fungi such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholera*, *Klebsiella pneumonia*, *Candida albicans* and *Cryptococcus neoformans* [43]. Furthermore, seeds of *S. lychnophora* inhibited the growth of *Streptococcus mutans* which is responsible for the formation of dental plaque and dental caries [44].

The notable activity of the total extract and ethyl acetate fraction of *B. rupestris* against Gram-negative bacteria was attributed to their richness in phenolic constituents such as flavonoids. Various flavonoids including quercetin, kaempferol, isorhamnetin and their glycosides were reported to be isolated from the leaves of *B. rupestris* [17]. Flavonoids are known to protect plants against microbial invasion

by plant pathogens [2]. Different flavonoids are known to possess antibacterial activity. Quercetin, kaempferol and their glycosides were reported to exhibit antibacterial activity [45]. The activity of quercetin has been attributed to the inhibition of DNA gyrase leading to the inhibition of nucleic acid synthesis.

The antifungal activity of *B. rupestris* *n*-hexane fraction might be correlated with the non-polar constituents such as sterols and triterpenes which were isolated from different members of the genus [11,12]. Sterols and triterpenes were stated to possess antifungal activity [46-48]. A study suggested that the antifungal activity of triterpenes was due to membrane lipid alteration which resulted in a change of membrane permeability [49].

Flavonoids as quercetin, rhamnetin, and quercetin-3-O-L-rhamnoside were isolated from the ethyl acetate fraction of *B. luridum* leaves [11]. These compounds are most probably responsible for the activity of this fraction against Gram-positive bacteria, *Staphylococcus aureus*. Meanwhile, the effect of *B. luridum* dichloromethane fraction of the leaves against Gram-negative bacteria such as *Escherichia coli* was suggested to be due to triterpenes which were reported before such as  $\beta$ -amyrin,  $\beta$ -amyrin acetate, lupeol and oleanolic acid [11]. Oleanolic acid, for example, was reported to exhibit antibacterial activity against Gram-negative bacteria by alteration of bacterial shape and inhibition of bacterial growth and survival [50]. Lupeol was reported in another study to exhibit antibacterial activity against *Escherichia coli* [51]. The activity of *B. luridum* total extract against *Salmonella typhi* might be due to the synergistic action of all compounds isolated from the plant such as triterpenes and flavonoids [11].

## Conclusion

In conclusion, our results revealed that the leaves of *B. rupestris* exhibited moderate antimicrobial activity against Gram-negative bacteria and fungi while the leaves of *B. luridum* showed notable activity against Gram-positive bacteria and moderate activity against Gram-negative bacteria. The phytochemical screening of both plants showed the presence of flavonoids, sterols, and triterpenes which might be correlated to their antimicrobial activity. These results confirm the traditional use of medicinal plants in infectious diseases. Further studies are ongoing in our group to identify compounds responsible for this antimicrobial activity.

## Declaration of Interest

The authors have declared no conflicts of interest.

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