Evaluation of Antimicrobial Activity of *Bauhinia purpurea* Leaves Under In Vitro Conditions

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Abstract This study was undertaken with an objective of testing the antibacterial and antifungal activities of *Bauhinia purpurea* leaves and identifying the bioactive compounds. The antimicrobial activity of leaf extract was determined in aqueous and organic extracts and the minimum inhibitory concentration (MIC) against six species of pathogenic and non-pathogenic microorganisms: *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* using the disk diffusion method. The chemical constituents of organic plant extract were separated by thin layer chromatography and purified by column chromatography and further identified by gas chromatography–mass spectrometry (GC–MS) analysis. Significant inhibitory activity was observed with methanol extracts of plant against the test microorganisms while less antibacterial activity was observed in hexane, acetone and aqueous extracts. MIC of *B. purpurea* extract was $\leq 1,500$ $\mu\text{g/ml}$ against *S. aureus* and *B. subtilis* while this extract showed no inhibition against Gram-negative *S. typhi*, *E. coli* and *P. aeruginosa* or against fungus *C. albicans*. Eleven compounds were identified in *B. purpurea* leaf extract by GC–MS analysis. The composition of *B. purpurea* revealed the presence of lupeol, stigmasterol, lanosterol, ergosterol, beta-tocopherol, phytol, hexadeconic acids, hexadeconic acids methyl esters, octadecadienoic acids and octadecatrienoic acid. Stigmasterol and lupeol were the most abundant (34.48 and 15.63 %). Other phytosterols like lanosterol (4.15 %) and ergosterol (2.82 %) were also found to be present in this extract.

Keywords Antimicrobial activity · Medicinal plants · MIC · TLC · GC–MS

Introduction

Plants have been used for centuries as a remedy for human diseases because they contain components of therapeutic value. They are natural sources of antimicrobial agents primarily because of the large biodiversity of such microorganisms and the relatively large quantity of metabolites that can be extracted from these plants [1]. The acceptance of these traditional medicines as an alternative form of health care has led researchers to investigate the antimicrobial activity of medicinal plants. Modern science has identified several secondary metabolites of various plant species that contain antimicrobial properties. Human infections constitute a serious problem and the most frequent pathogens are microorganisms such as bacteria and fungi. Therefore, the search for discovery of new antimicrobial agents is necessary and stimulates the research for new chemotherapeutic agents in medicinal plants [2, 3].

Bauhinia purpurea Linn. (Leguminosae) commonly known as purple orchid tree is a medium sized deciduous tree, sparingly grown in India. It is used in gastrointestinal activities with applications as a laxative (flowers), a carminative drug (roots) and for diarrhea (bark). Traditionally this plant is used in the treatment of dropsy, pain, rheumatism, convulsion, delirium, septicaemia, etc. [4]. The aerial parts of the plant are reported to contain flavone glycosides, dimeric flavonoids, 6-butyl-3-hydroxy flavanone, amino acids, phenyl fatty ester, lutine and β -sitosterol [5]. *B. purpurea* is now used as a new paraffin section marker for Reed–Sternberg cells of Hodgkin’s lymphoma [6]. *B. purpurea* lectin (BPA) was purified from seeds of

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B. purpurea alba. BPA is a Gal β 1-3GalNAc (T) specific leguminous lectin that has been widely used in multifarious cytochemical and immunological studies of cells and tissues under pathological or malignant conditions [7]. BPA is also used as a marker in hyperblastic human tonsil and peripheral blood mononuclear cells by immunohistological, immunoelectron microscopic, and flow cytometric techniques [8].

The aim of the present study was to identify the active components responsible for the antimicrobial activity in *B. purpurea* with a view of ascertaining their possible medicinal values. The antimicrobial activity of the *B. purpurea* leaf extract was done by agar well diffusion and twofold serial dilution techniques. For the identification of compounds, the extract was purified by column chromatography and subjected to GC–MS analysis.

Materials and Methods

Plant Material

The major raw material used in this work was freshly harvested *B. purpurea* leaves collected during January 2008 from National Gir Foundation, Gandhinagar, Gujarat with the assistance of local plant keepers and authenticated by officials of the Gir Foundation. Fresh leaves were washed under running tap water followed by sterilized distilled water, shade dried and then powdered with the help of a sterilized pestle and mortar.

Aqueous Extract Preparation

Shade dried fine powdered leaves (10 g) were boiled in 400 ml distilled water till one-fourth of the extract initially taken was left behind after evaporation. The solution was then filtered using sterile muslin cloth. The filtrate was centrifuged at 5,000 rpm for 15 min. The supernatant was again filtered using Whatman filter paper no. 1 under strict aseptic conditions. The filtrate was collected in sterilized bottles and stored at 4 °C until further use.

Organic Solvent Extract Preparation

Shade dried powder (10 g) was thoroughly mixed with 100 ml organic solvent (methanol, hexane or acetone). The mixture was placed at room temperature for 24 h on a shaker at 150 rpm. The solution was filtered through muslin cloth and then re-filtered by passing through Whatman filter no. 1. The filtrate thus obtained was concentrated by complete evaporation of the solvent at room temperature to yield the pure extract. Stock solutions of crude extracts for each type of organic solvent (methanol, hexane or acetone)

were prepared by mixing well an appropriate amount of dried extracts with dimethyl sulphoxide (DMSO) to obtain a final concentration of 100 mg/ml that was used for the evaluation of antibacterial and antifungal activities. Each solution was stored at 4 °C after collecting in sterilized bottles until further use.

Microbial Cultures

The test microorganisms used for the antimicrobial activity screening were *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 10707), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC 10231). All microorganisms were obtained from the microbiology laboratory at the Department of Life Sciences and MG Institute of Science at Gujarat University. All the cultures were maintained on nutrient agar (NA) medium.

Culture Media

Mueller–Hinton agar (MHA, 17.5 g casein hydrolysate, 3.0 g beef extract, 1.5 g starch and 15 agar/l), sabouraud dextrose agar (SDA, 20 g glucose, 10 g peptone and 20 g agar/l) and NA (3.0 g beef extract, 3.0 g NaCl, and 5.0 g peptone/l) were used for microbiological studies.

Antibacterial Assay

In vitro antibacterial activities of all aqueous and organic extracts of *B. purpurea* were determined by standard agar well diffusion assay [9]. Petri dishes (100 mm) containing 25 ml of MHA were seeded with 100 μ l inoculum of the test strain (inoculum size was adjusted so as to deliver a final inoculum of approximately 10^6 CFU/ml) and allowed to solidify. Wells of 6 mm diameter were cut into solidified agar media with the help of a sterilized cork-borer. 100 μ l of each extract was poured in the respective well and the plates were incubated at 37 °C for 24 h. DMSO and sterilized distilled water were used as negative controls while tetracycline antibiotic (1 U) was used as a positive control. The experiment was performed in triplicate under strict aseptic conditions and the antibacterial activity of each extract was expressed in terms of the mean diameter of zone of inhibition (mm) produced by the respective plant extract.

MIC for the Bacteria

The antibacterial activity of the extracts was examined by determining the minimum inhibitory concentration (MIC) in accordance with Clinical and Laboratory Standard Institute (CLSI) methodology [10]. All tests were carried

out in Mueller–Hinton broth (MHB, 17.5 g casein hydrolysate, 3.0 g beef extract and 1.5 g starch) supplemented with DMSO at a final concentration of 10 % (v/v) in order to enhance their solubility. The plant extract were dissolved in MHB. Test strains were suspended in MHB to give a final density of 5×10^5 CFU/ml and confirmed by viable counts. Dilutions ranging from 100 to 2,000 mg/ml of the plant extract were prepared in test tubes including one growth control [MHB + 10 % DMSO (v/v)] and one sterility control [MHB + 10 % DMSO (v/v) + test extracts]. The MIC values were determined from visual examination as being the lowest concentration of the extracts with no bacterial growth. Test tubes were incubated at 37 °C for 24 h.

MIC for the Yeast

The antifungal activity of the extracts was examined by determining the MIC in accordance with CLSI methodology [11] using yeast nitrogen base glucose (YNBG) medium (YNB 6.7 g, monohydrated glucose 20 g/l) supplemented with DMSO at a final concentration of 10 % (v/v). The extracts were dissolved in YNBG medium. Yeast strains were cultured for 24–48 h at 35 °C on SDA and then suspended in 4 ml of sterile distilled water by adjusting to 1 McFarland using a nephelometer to give a final inoculum concentration of $1.5 \pm 1.0 \times 10^3$ CFU/ml. Dilutions ranging from 100 to 2,000 mg/ml of the extracts were prepared in the tubes including one growth control [YNBG + 10 % DMSO (v/v)] and one sterility control [YNBG + 10 % test extracts (v/v)]. A 100 µl suspension of *Candida* strain in YNBG was added and incubated at 35 °C for 48 h. The MICs of the extracts were defined as the lowest concentration that inhibited 80 % of visible fungal growth. The final concentration of DMSO in the assays did not interfere with either the bacterial or candidal proliferation.

Statistical Analysis of Data

The experiments were laid out according to randomized block design (for single factor experiments) or nested design (two factor experiments). Each zone of inhibition experiment had three replicates and the mean of three replicates was reported. The analysis of variance (ANOVA) appropriate for the design was carried out to detect significance of difference among the treatment means.

Separation of Phytochemicals by Bioautography

The dried leaves (250 g) were powdered and macerated with methanol for 1 week in a shaker. The collected extracts were filtered and evaporated under vacuum. The residues were dried and weighted.

Thin Layer Chromatography of Plant Extract

Thin layer chromatography (TLC) technique was employed as a means of assessing quality and purity of plant extract [12]. TLC of the extract was made by using different solvents and combinations of solvents. Methanol, chloroform, toluene, petroleum ether and ethyl acetate singly and in combination were used as developing agents.

Column Chromatography Separation

Wet-process method was adopted to pack the column. Silica gel acted as a sorbent and mixture of petroleum ether and ethyl acetate (80:20) and toluene and ethyl acetate (80:20) one by one were the eluents. At the beginning, silica gel was added to the eluent. It was stirred and slowly poured into the column until the bed layer was solid. The temperature of the column was 25 °C. The petcock was opened to decrease eluent level until its liquid surface and silica gel level were equal. The sample solution was poured slowly into the column along its wall. Finally, the eluent was added to the column, keeping the sorbent covered by the eluent. For both mixtures, five samples were collected in test tubes.

GC–MS Analysis

For GC–MS analysis, a Hewlett–Packard-5890-II (Global Medical Instrumentation) gas chromatograph, equipped with a flame-ionization detector (FID) and coupled with an electronic integrator was used. Quantitative data were obtained by electronic integration of the FID-area data, without response factor correction.

Results and Discussion

The results of aqueous and organic plant extracts as determined by agar diffusion method are presented in Table 1. The results indicate that the aqueous extract was found to be ineffective as it did not exhibit zones of inhibition with the test strains. Amongst organic extracts, methanol extract showed promising activity against the test isolates. The antimicrobial activity in terms of zone of inhibition for methanol extract was 18 ± 0.18 mm for *S. aureus*, 20 ± 0.16 mm for *B. subtilis*, 18 ± 0.18 mm for *E. coli*, 17 ± 0.16 mm for *S. typhi*, 18 ± 0.14 mm for *P. aeruginosa* and 18 ± 0.16 mm for *C. albicans*. The zone of inhibition for hexane extract was 11 ± 0.12 mm for *S. aureus*, 10 ± 0.10 mm for *B. subtilis*, 11 ± 0.12 mm for *E. coli*, 11 ± 0.12 mm for *S. typhi*, 10 ± 0.10 mm for *P. aeruginosa* and 12 ± 0.16 mm for *C. albicans*. The zone of inhibition for acetone extract was

Table 1 Results of antimicrobial screening of aqueous and organic plant extracts determined by agar diffusion method

Plant type	Extraction type (mg/ml)	Zone of inhibition (in mm diameter)					
		<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
<i>Bauhinia purpurea</i>	Methanol	18 ± 0.18	20 ± 0.16	18 ± 0.18	17 ± 0.16	18 ± 0.14	18 ± 0.16
	Hexane	11 ± 0.12	10 ± 0.10	11 ± 0.12	11 ± 0.12	10 ± 0.10	12 ± 0.16
	Acetone	10 ± 0.10	10 ± 0.10	11 ± 0.14	10 ± 0.10	11 ± 0.16	11 ± 0.12
	Aqueous	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	ND
Positive control	Tetracycline	22 ± 0.26	22 ± 0.26	20 ± 0.24	22 ± 0.28	20 ± 0.24	22 ± 0.24
Negative control	DMSO	NA	NA	NA	NA	NA	NA

Zone of inhibition (in mm diameter) including the diameter of well (6 mm) in agar well diffusion assay. Assay was performed in triplicate and results are the mean of three values. In each well, the sample size was 100 µl. Tetracycline: one unit strength

ND not detected, NA no activity

10 ± 0.10 mm for *S. aureus*, 10 ± 0.10 mm for *B. subtilis*, 11 ± 0.14 mm for *E. coli*, 10 ± 0.10 mm for *S. typhi*, 11 ± 0.16 mm for *P. aeruginosa* and 11 ± 0.12 mm for *C. albicans* as compared to the positive control (tetracycline) which is 22 ± 0.26 mm for *S. aureus*, 22 ± 0.26 mm for *B. subtilis*, 20 ± 0.24 mm for *E. coli*, 22 ± 0.28 mm for *S. typhi*, 20 ± 0.24 mm for *P. aeruginosa* and 22 ± 0.24 mm for *C. albicans*.

Among all the bacterial strains tested, *B. subtilis* was found to be the most susceptible with a maximum inhibition by the extract prepared in methanol producing a zone of inhibition ≥20 mm (Table 1). Extracts prepared in organic solvents consistently displayed better antimicrobial activity than that of aqueous extracts. Furthermore, extracts prepared in methanol showed the most inhibition followed by those prepared in hexane and acetone, respectively. The present study indicates that under the experimental conditions, methanol is an ideal solvent to extract antimicrobial compounds from leaves. Polyphenolic compounds such as flavonols and most other reported bioactive compounds are generally soluble in polar solvents such as methanol [13]. Most antimicrobial active components that have been identified are not water soluble and thus organic solvent extracts have been found to be more potent. The inhibitory effects on test microorganisms are mainly due to plant extract. Plant samples were first extracted in these buffers and after extraction all the solvents were evaporated and

only thick plant residue was left which was then made to the final volume by adding DMSO. In the present study plant powder was sequentially extracted with different solvents in increasing polarity order. It was found that the polarity of the solvents seems to play an important role in the extraction of natural products which influences the antimicrobial activity of the extracts [14].

B. purpurea leaf extract prepared in methanol was subjected to determination of the MIC by standard twofold macrobroth dilution method against the test organisms. All antimicrobial activity occurred in a concentration dependent manner as suggested by the MIC determination. However, the efficacy of extracts was less than the standard antibiotic, tetracycline. The MIC of *B. purpurea* extract was ≤1,500 µg/ml against *S. aureus* and *B. subtilis* while this extract shows no inhibition against Gram-negative *S. typhi*, *E. coli* and *P. aeruginosa* or against the fungus *C. albicans* (Table 2).

The present study shows that *B. purpurea* leaf extract was only effective against Gram-positive *S. aureus* and *B. subtilis*. No antimicrobial activity was recorded against Gram-negative *E. coli*, *S. typhi* and *P. aeruginosa* or against the fungus *C. albicans*. The results are in accordance with the antimicrobial tests in which it was reported that phytol fatty esters present in *B. purpurea* leaves had low activity against the fungi, *A. niger* and *C. albicans* and are inactive against the bacteria, *P. aeruginosa* and *E. coli*.

Table 2 Results of minimum inhibitory concentration (MIC) of methanol extract of test plant

Plant type	Extraction type	Minimum inhibitory concentration (mg/ml)					
		<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
<i>Bauhinia purpurea</i>	Methanol	150	150	NI	NI	NI	NI
Positive control	Broth + TO	G	G	G	G	G	G
Negative control	Broth + CE	NG	NG	NG	NG	NG	NG

TO test organisms, CE crude extract, G growth, NG no growth, NI no inhibition

Microorganisms: *S. aureus*, *Staphylococcus aureus*; *B. subtilis*, *Bacillus subtilis*; *E. coli*, *Escherichia coli*; *S. typhi*, *Salmonella typhi*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *C. albicans*, *Candida albicans*

In general, the cell envelope of Gram-negative organisms, which is more complex than Gram-positive ones, acts as a diffusional barrier and makes them less susceptible to the antimicrobial agents [15].

Bioautography overcomes the challenge of isolating antimicrobial compounds from crude extracts with complex chemical components by simplifying the process of their isolation and identification. It uses very little sample which is ideal for plant extracts [16]. *B. purpurea* leaf extract prepared in methanol was subjected to purification by column chromatography with a combination of solvents (i.e., petroleum ether and ethyl acetate, toluene and ethyl acetate, petroleum ether and chloroform). Previously when a single solvent system had been used no elution resulted. From eluent A (petroleum ether and ethyl acetate ratio 40:60) fraction 2 showed a clear spot with a R_f value 0.48 and from eluent B (petroleum ether and ethyl chloroform ratio 40:60) fraction 2 showed a clear spot with a R_f value 0.86. The R_f value of a compound determined under identical conditions is a characteristic of each compound and can be used as an aid to its identity. TLC purification of these two fractions was done and was used for GC–MS analysis to find out the nature of the compounds responsible for the antimicrobial activity.

The results of GC–MS analysis showed that the *B. purpurea* leaf extract contained a total of 11 identified compounds based on their retention times (RT) and retention indexes (RI). These compounds were identified through mass spectrometry attached with GC. The mass spectra of these compounds were matched with those found in the NIST/NBS spectral databases. Some of these compounds were not identified and all of these were present in concentration of less than 2 % as auto quantified by the GC–MS machine. Lupeol, stigmasterol, lanosterol, ergosterol, beta-tocopherol, phytol, hexadeconic acid, hexadeconic acid methyl esters, octadecadienoic acids and octadecatrienoic acid were the major components of this extract, with stigmasterol and lupeol as the most abundant (34.48 and 15.63 %). Other phytosterols like lanosterol (4.15 %) and ergosterol (2.82 %) were also found to be present in this extract as shown in Table 3. It can be concluded that the compound with a RT = 57.4, base peak 9,992 and area % 15.63 is lupeol; the compound with RT = 56.3, base peak 23,453 and area % 34.48 is stigmasterol; the compound with RT = 58.5, base peak 3,599 and area % 4.15 is lanosterol; the compound with RT = 53.4, base peak 3,576 and area % 2.82 is ergosterol; the compound with RT = 52.8, base peak 12,885 and area % 2.73 is beta-tocopherol; and the compound with RT = 38.5, base peak 27,623 and area % 4.93 is phytol.

On the basis of the present investigation, it can be highlighted that the antimicrobial activity of *B. purpurea* leaf extract might be correlated to the presence of

Table 3 Compounds identified in the *B. purpurea* leaf extract

No	Compound name	Retention time (RT)	Amount (%)
1	Hexadecanoic acid, methyl ester	34.958	1.93
2	Hexadecanoic acid	35.814	9.53
3	9,12-Octadecadienoic acid (Z,Z) methyl	38.164	2.56
4	9,12,15-Octadecatrienoic acid, methyl	38.292	3.83
5	Phytol	38.505	4.93
6	Beta-tocopherol	52.808	2.73
7	Ergost-5-en-3-ol, acetate	53.441	2.82
8	Vitamin E acetate	53.866	17.41
9	Stigmast-5-en-3-ol	56.274	34.48
10	Lupeol	57.387	15.63
11	Lanosterol	58.462	4.15

flavonoids and phenolic compounds. The mechanism of antimicrobial activity is complicated and could be attributed to synergism between flavonoids, hydroxy acids and sesquiterpenes [17]. Flavonoids are the largest group of secondary metabolites in plants. Flavonoids exhibit antimicrobial activity through formation of a complex with the bacterial cell wall. They also possess antioxidant activity due to presence of a phenolic ring in the moiety [18, 19].

The present study evaluated the development of the medicinal plant *B. purpurea* which exhibited antibacterial activities against two Gram-positive bacteria (*S. aureus* and *B. subtilis*). The findings are consistent with earlier reports in which *Bauhinia* species containing anthraquinone, flavonoids and polysaccharides that showed considerable activity against Gram-positive bacteria. The results are considered important since *S. aureus* is an important pathogen in man and animals and where resistance to other drugs is frequently reported. Methicillin resistant *S. aureus* is widely distributed among hospitals and increasingly isolated from community-acquired infections [20].

Antimicrobials of plant origin have enormous therapeutic potential and have been proven effective in the treatment of infectious diseases simultaneously mitigating many of the side effects which are often associated with synthetic antibiotics [21]. Positive response of plant based drugs might lie in the structure of natural products which react with toxins and/or pathogens in such a way that less harm is done to other important molecules or physiology of the host. Plants and natural products remain as an untapped reservoir of potentially useful chemical compounds not only as drugs but also as unique templates that could serve as a starting point for synthetic analogs. The leaf extract of *B. purpurea* has potential as an antibacterial agent so this plant can potentially be used in the treatment of infectious diseases caused by microorganisms that show resistance to currently available antibiotics. The present study suggests

that further study of this plant extract for its therapeutic efficacy is essential for the isolation of active fractions.

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References

- Copp BR (2003) Antimycobacterial natural products. *Nat Prod Rep* 20:535–557
- Kaushik P, Dhiman AK (2000) Medicinal plants and raw drugs of india. Bishen Singh Mahendra Pal Singh, New Cannaught Place
- Kim IG, Kang SC, Kim KC, Choung ES, Zee OP (2008) Screening of estrogenic and antiestrogenic activities from medicinal plants. *Environ Toxicol Pharmacol* 25(1):75–82
- Yadava RN, Tripathi P (2000) A novel flavone glycoside from the stem of *Bauhinia purpurea*. *Fitoterapia* 71(1):88–90
- Pettit GR, Numata A, Iwamoto C, Usami Y, Yamada T, Ohishi H (2006) Isolation and structures of *Bauhinia* statins 1–4 from *Bauhinia purpurea*. *J Nat Prod* 69:323–327
- Wu AM, Wu JH, Jia-Hua Liu, Tanuja S (2004) Recognition profile of *Bauhinia purpurea* agglutinin (BPA). *Life Sci* 74(14): 1763–1779
- Sarkar AB, Akagi T, Yoshino T, Fujiwara K, Murakami I (1993) *Bauhinia purpurea* lectin (BPA) binding spectra in hyperplastic human tonsil and in peripheral blood: immunohistochemical, immunoelectron microscopic and flow cytometric analysis. *J Histochem Cytochem* 41(6):67–72
- Sarkar AB, Akagi T, Jeon HJ, Miyake K, Murakami I, Yoshino T, Takahashi K, Nose S (1992) *Bauhinia purpurea*—a new paraffin section marker for Reed–Sternberg cells of Hodgkin’s disease. A comparison with Liu-M1 (CD15), LN2 (CD74), peanut agglutinin and Ber-H2 (CD30). *Am J Pathol* 141:19–23
- Smith P, Douglas I, McMurray J, Carroll C (2009) A rapid method of improving the criteria being used to interpret disc diffusion antimicrobial susceptibility test data for bacteria associated with fish diseases. *Aquaculture* 290(1–2):172–178
- Clinical and Laboratory Standards Institute CLSI (2005) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th edn.: approved standard M7-A6 CLSI. Wayne, Pennsylvania
- Clinical and Laboratory Standards Institute CLSI (2008) Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard, 3rd edn. M27-A3 CLSI, Wayne, Pennsylvania
- Nostro A, Germano MP, Angelo VD, Marino A, Canatelli MA (2000) Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett Appl Microbiol* 30: 384–397
- Parekh J, Jadeja D, Chanda S (2005) Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turk J Biol* 29:203–210
- Neto MM, Neto MA, Filho RB, Lima MAS, Silveira ER (2008) Flavonoids and alkaloids from leaves of *Bauhinia unguolata* L. *Biochem Syst Ecol* 36(3):227–229
- Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. *Int J Food Microbiol* 94:223–253
- Runyoro DKB, Matee MIN, Ngassapa OD, Joseph CC, Mbwapo ZH (2006) Screening of Tanzanian medicinal plants for anti-candida activity. *BMC Complement Altern Med* 6(11): 1186–1472
- Vardar-Unlu G, Silici S, Unlu M (2008) Composition and in vitro antimicrobial activity of *Populus* buds and poplar-type propolis. *World J Microbiol Biotechnol* 24:1011–1017
- Ragasa CY, Hofilena J, Rideout JA (2004) Secondary metabolite from *Bauhinia purpurea*. *Ind J Microbiol* 133(1):1–5
- Mikamo E, Okada Y, Semma M, Ito Y, Morimoto T, Nakamura M (2000) Studies on structural correlation with antioxidant activity of flavonoids. *Jpn Soc Food Chem* 7(2):93–97
- Chambers HF, Merle AS (1996) Antimicrobial agents—general considerations. In: Joel GH, Lee EL (eds) Goodman and Gilman’s the pharmacological basis of therapeutic, 9th edn. McGraw-Hill, Medical Publishing Division, New York
- Iwu MW, Duncan AR, Okunji CO (1999) New antimicrobials of plant origin. In: Janick J (ed) Perspectives on new crops and new uses. ASHS Press, Alexandria, pp 457–462