ORIGINAL ARTICLE

Phytochemical analysis and antibacterial activity of *Acacia nilotica* (L.) leaves against pathogenic bacteria

Rupal Singh[1], Rupesh Thakur[2]

[1]Biochemical Research Laboratory, Centre for Scientific Research and Development, People's University, Bhopal, Madhya Pradesh, India, [2]Department of Biotechnology, St. Aloysius College (Autonomous), Jabalpur, Madhya Pradesh, India

Abstract

Aim: To evaluate the phytochemical profile and *in vitro* antibacterial activity of leaves of *Acacia nilotica* against pathogenic bacteria. **Materials and Methods:** The crude extracts were prepared by two methods separately with three different solvents and examined for the preliminary phytochemical screening and antibacterial activity using agar well diffusion assay. On the basis of the preliminary analysis, solvent fractionation of the best crude extract was done employing two sets of solvents, and the solvent fractions were finally subjected to antibacterial activity. The quantitative analysis of total phenolics and total flavonoids content in the crude methanolic extract and solvent fractions were also done. **Results:** The crude extract and solvent fraction showed varying degree of inhibitory activity against tested bacteria. The result of antibacterial activity revealed more susceptibility toward Gram-positive bacteria as compared to Gram-negative bacteria. **Conclusion:** The most active solvent extract or fraction can be further explored to isolate and characterize the bioactive components responsible for biological activity to develop new antibacterial drugs.

Key words: *Acacia nilotica*, antibacterial activity, crude extracts, phytochemical analysis, solvent fractionation

INTRODUCTION

Natural sources, such as plants and their products, have been used in the indigenous system of medicine from time immemorial for curing diseases. Being a rich source of secondary metabolites such as phenolic acids, flavonoids, tannins, alkaloids, and other small compounds, plants can be of interest in therapeutics. Various plant extracts and phytochemicals offer considerable potential for the development of new agents effective against infections and could help curb the problem of multidrug-resistant organisms.[1]

The genus *Acacia* belongs to the family Leguminosae. It is a cosmopolitan genus containing more than 1350 species, distributed throughout tropical and warm temperate areas of the world.[2] Out of these species, *Acacia nilotica* (also known as Gum Arabic tree, Babul, Egyptian thorn, or Prickly Acacia) is widely cultivated in the Indian subcontinent and also found on lateritic soil in the Himalayan foothills.[3] This multipurpose nitrogen fixing tree legume occurs from sea level to over 2000 m and withstand at extreme temperature (>50°C) and air dryness.[4] Traditionally, the plant is used widely for the treatment of various ailments, but scientifically few of them were screened out.[3] It is used as an antiseptic, demulcent, purgative,[5] and an effective tonic in diabetes mellitus.[6] Several species of *Acacia* have been proven as an effective medicine in the treatment of a cough, toothache, diarrhea, dysentery, jaundice, and skin disorders.[7-10] Beside this, its various parts possess significant antibacterial and antifungal properties.[11,12] *A. nilotica* is also reported to be effective against multidrug-resistant strains of bacteria and fungus causing nosocomial and community-acquired infections.[13] Phytochemical screening of different parts of *A. nilotica* showed distinct classes of secondary metabolites having

Address for correspondence:
Rupal Singh, Biochemical Research Laboratory, Centre for Scientific Research and Development, People's University, Bhopal - 462 037, Madhya Pradesh, India. E-mail: rupalsingh86@gmail.com

Received: 20-01-2016
Revised: 24-04-2016
Accepted: 28-04-2016

therapeutic potential.[14,15] The phytochemical analysis of leaf extract of *A. nilotica* revealed the presence of several biological active compounds such as 3-picoline-2-nitro, 1-acetyl beta-carboline, hydroxycitronellal, trans decalone, propionic acid-2-chloro, ethyl ester, lavandulyl acetate, and D-glucuronic acid by gas chromatography-mass spectrometry analysis.[16]

A considerable body of literature deals with the antibacterial activity of this plant and their extracts against a wide variety of Gram-positive and Gram-negative bacteria from distinct regions of India,[17-19] but very few studies has been done on plants of Central India origin. Probably, the same species of plant belonging to different regions show different antibacterial activity and phytochemical composition because of the geographical variations. So, it is important to reveal the antibacterial activity of plants from Central India origin. Apparently, none of the studies were performed on various solvent fractions obtained from solvent fractionation so far. Considering this, a systematic investigation was undertaken, in which leaves of *A. nilotica*, prevalent in Central India, were screened for *in vitro* antibacterial activity and phytochemical analysis.

MATERIALS AND METHODS

Plant Material and Bacteria

The leaves of *A. nilotica* were collected from the local area of Bhopal, Madhya Pradesh, India. Fresh and healthy leaves were chosen, washed thoroughly, and shade-dried at ambient temperature. The dried leaves were then crushed to fine powder of 60-mesh size and stored at 4°C until further use. A total of 10 different ATCC bacteria were used for the antibacterial activity assay. These were maintained on desired media agar slants, stored at 4°C and sub-cultured periodically.[20]

Phytoconstituents Extraction and Antibacterial Activity

The phytoconstituents extraction was done by cold (infusion) and hot (Soxhlet) solvent extraction separately with three solvents, viz., methanol, ethanol, and butanol. The percentage yield of each extract was calculated, and the dried extracts were stored airtight at 4°C for further use. The *in vitro* antibacterial activity of three hot and three cold extracts at 100 mg/ml was performed by agar well diffusion assay on Mueller-Hinton Agar (MHA) medium. Chloramphenicol and ampicillin at a concentration of 0.5 mg/ml were used as positive controls and blank dimethyl sulfoxide (DMSO) as a negative control. The experiment was performed in triplicate for each bacteria and the antibacterial activity of each extract was expressed in terms of the mean of the diameter of the zone of growth inhibition in mm.[21]

Mass Extraction, Solvent Fractionation, and Antibacterial Activity

On the basis of the results of preliminary analysis of antibacterial activity, 200 g of fine leaf powder was Soxhlet extracted with 1000 ml of methanol to obtain the crude methanolic extract. It was dissolved in 20% methanolic water to make a crude extract solution (CES) for solvent fractionation employing two sets of solvents, namely, set-petroleum spirit, chloroform, and ethyl acetate (PCE) and set-hexane, dichloromethane, and butanol (HDB), which resulted in total, six organic and two aqueous fractions (AFs), *viz*., petroleum spirit fraction (PF), chloroform fraction (CF), ethyl acetate fraction (EF), hexane fraction (HF), dichloromethane fraction (DF), butanol fraction (BF) and remaining AF-I and AF-II, respectively. The percentage yield of each dried organic fractions (PF, CF, EF, HF, DF, and BF) and AF-I and AF-II was calculated and subjected to *in vitro* antibacterial activities at 25 mg/ml concentration by agar well diffusion assay on the MHA medium as described earlier.[21]

Phytochemical Analysis

The qualitative phytochemical screening of all the six crude extracts (cold and hot) was individually performed for the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, tannins, and terpenoids by the standard procedure.[17,22] Quantitative estimation of total phenol and total flavonoid content were also carried out.

Determination of total phenolic content

The total soluble phenolic compounds in the methanolic crude extracts and solvent fractions were determined with Folin-Ciocalteu reagent using gallic acid as a standard. The extract was diluted to a working concentration of 50 µg/ml in methanol. This soluted extract (0.5 ml) was mixed with 2.5 ml of Folin-Ciocalteu reagent (10%) and incubated for 5 min at room temperature. After that, 2 ml of sodium carbonate (7.5%) was added followed by incubation at room temperature for 1 h with intermittent shaking. Next, the samples were centrifuged at 5000 rpm for 1 min, and the absorbance of the supernatant was measured at 765 nm versus blank sample on a visible spectrophotometer. The samples were prepared in triplicate, and the mean value of absorbance was recorded.[23,24]

Determination of total flavonoid content

The total flavonoid content was determined spectrophotometrically according to the standard method. For this, 0.5 ml of working concentration (1 mg/ml) was mixed with 1.5 ml of methanol and 0.1 ml of 10% aluminum chloride solution followed by incubation for 5 min at room temperature. After this, 0.1 ml of 1 M potassium acetate was added and again incubated for 5 min at room temperature. Subsequently, 2.8 ml of distilled water was added and incubated for 1 h at room temperature. Absorption readings

at 415 nm were taken against a blank. The total flavonoid content was determined using a standard curve with quercetin. The samples were prepared in triplicate, and the mean value of absorbance was obtained.[23,25]

RESULTS AND DISCUSSION

Percentage Yield of Crude Extracts

The percentage yield of crude extracts after cold (infusion) and hot (Soxhlet) extraction methods was calculated and shown in Table 1. In case of cold extraction, the percentage yield of methanol extract was the highest (20.93%) followed by ethanol extract (19.41%) and butanol extract (17.02%). Similarly, with hot extraction, methanol extract resulted in the highest percentage yield (48.49%), followed by ethanol extract (35.65%) and butanol extract (26.78%). Overall, the percentage yield of extracts obtained with three different solvents by Soxhlet extraction was higher than the yield obtained from infusion method.

Preliminary Antibacterial Activity of Crude Extracts

The six crude extracts (three hot and three cold) were subjected to *in vitro* preliminary antibacterial bioassay at 100 mg/ml concentration against 10 different bacteria. The result showed varying degrees of inhibitions. The cold and hot extracts inhibited the growth of four test bacteria, *viz*., *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus epidermidis,* and *Streptococcus pyogenes* [Table 2]. On the other hand, another six bacteria, that is, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi,* and *Salmonella typhimurium* did not display any inhibition against any crude extract at administered concentration.

Table 1: Percentage yield of *A. nilotica* extracts prepared by two methods

Phytoconstituents extraction method	Solvent name	% Yield
Cold[a]	Butanol	17.02
	Ethanol	19.41
	Methanol	20.93
Hot[b]	Butanol	26.78
	Ethanol	35.65
	Methanol	48.49

[a]Infusion (cold extraction), [b]Soxhlet (hot percolation).
A. nilotica: Acacia nilotica

In case of the cold solvent extracts, the data showed that the highest values of the diameter of the zone of inhibition were exhibited by methanolic extract against *S. pyogenes* giving a zone diameter of 17.00 ± 0.05 mm, whereas the lowest antibacterial response was observed against *E. faecalis* with 7.33 ± 0.58 mm zone of inhibition. Among the hot solvent extracts, methanolic extracts exhibited the highest value of the zone of inhibition against *S. pyogenes* giving zone of diameter of 16.83 ± 0.29 mm. Butanolic extract exhibited the lowest value of inhibition zone against *B. subtilis* with 5.00 ± 0.00 mm zone of inhibition [Table 2]. Positive controls, chloramphenicol, and ampicillin showed variable inhibition diameters against Gram-positive and Gram-negative bacteria, whereas DMSO solvent, i.e., the negative control used for the extract preparation showed no inhibitory activity against any bacteria, indicating that the plant extract itself and not solvent inhibited the growth of the test bacteria.

In contrast, the previous study reported that some bacterial strains, *viz*., *S. typhimurium*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa* were sensitive to ethanolic extracts of *A. nilotica,* and the minimum inhibitory concentration (MIC) value against different isolates was found to be in the range of 4.9-313 µg/mL.[13] Another study revealed concentration-dependent sensitivity of bacterial pathogens against hot aqueous extract of *A. nilotica*. The extract produced dose-dependent zone of inhibition against *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *B. cereus*, *S. aureus,* and *S. uberis*.[11] However, similar to our study, *K. pneumoniae* showed marked resistance toward ethanol and chloroform leaves extracts of *A. nilotica*. The extracts exhibited considerable bacteriostatic activity against two Gram-positive and three Gram-negative strains with maximum zone of inhibition of 29 mm diameter

Table 2: Antibacterial activity of cold and hot extracts of A. *nilotica*

Bacterial cultures	Herbal extract (100 mg/ml)					
	Butanolic		Ethanolic		Methanolic	
	Cold[a]	Hot[b]	Cold[a]	Hot[b]	Cold[a]	Hot[b]
B. subtilis	9.33±0.29	5.00±0.00	8.50±0.00	5.83±0.29	9.00±0.00	6.67±0.58
E. faecalis	7.33±0.58	6.17±0.29	7.83±0.29	9.33±0.29	8.50±0.00	9.33±0.58
S. epidermidis	9.50±0.00	7.67±0.58	11.17±1.26	8.67±0.58	9.50±0.50	8.33±0.58
S. pyogenes	15.17±0.29	9.67±0.29	15.33±0.29	16.67±0.29	17.00±0.05	16.83±0.29

Antibacterial activity was expressed in terms of diameter of zone of growth inhibition in mm (mean±SD, n=3). [a]Infusion (cold extraction), [b]Soxhlet (hot percolation), *B. subtilis: Bacillus subtilis, E. faecalis: Enterococcus faecalis, S. epidermidis: Staphylococcus epidermidis, S. pyogenes: Streptococcus pyogenes*, SD: Standard deviation, *A. nilotica: Acacia nilotica*

against *E. coli* while a minimum 8 mm zone of inhibition against *B. subtilis*.[26]

On the whole, the antibacterial screening process of this study showed that the crude methanolic extract of *A. nilotica* showed appreciable inhibitory activity followed by ethanolic and butanolic extracts. This is in accordance with a previous study, in which the antibacterial activities of methanolic and aqueous extracts of *A. nilotica* were evaluated against clinically important pathogens. Among the two extracts, the methanol was found to be most active against all the tested bacterial species except *S. aureus*. The MICs of the extracts ranged between 0.125 and 2 mg/ml (*K. pneumoniae* [0.125 mg/ml], *B. subtilis* [0.500 mg/ml], *E. coli* [2.0 mg/ml], *P. vulgaris* [2.0 mg/ml], *P. aeruginosa* [1.0 mg/ml]).[27]

Mass Extraction and Solvent Fractionation

On the basis of results obtained from the preliminary analysis, it was found that the Soxhlet extraction gave a higher percentage yield of crude extracts, and the methanolic extract was considered to be the most active in respect to the wide range of inhibition zones against all test bacteria. Therefore, hot extraction (Soxhlet) procedure using methanol as suitable solvent was selected for the mass extraction of crude phytoconstituents extract. The CES, after partitioning separately with set-PCE and set-HDB system, yielded six solvent and two AFs. The percentage yields of all the fractions were calculated, and it was found that in set-PCE, the highest percentage yield was obtained from EF (43.96%) followed by AF-I (19.42%), PF (0.81%), and CF (0.61%). Similarly, in set-HDB, BF yielded highest value (41.51%) following AF-II (17.54%), DF (0.40%), and HF (0.22%) [Table 3].

Table 3: Percentage yield of solvent fractions obtained with two set of solvents

Solvent set	Fraction	% Yield
Set-PCE	PF	0.81
	CF	0.61
	EF	43.96
	AF-I	19.42
Set-HDB	HF	0.22
	DF	0.40
	BF	41.51
	AF-II	17.54

PCE: Petroleum spirit, chloroform, and ethyl acetate, HDB: Hexane, dichloromethane, and butanol, AF: Aqueous fractions, PF: Petroleum spirit fraction, CF: Chloroform fraction, EF: Ethyl acetate fraction, HF: Hexane fraction, DF: Dichloromethane fraction, BF: Butanol fraction

Antibacterial Activity of Solvent Fractions

The antibacterial activity of six solvents and two AFs of *A. nilotica* at 25 mg/ml concentration followed different trends as compared to preliminary antibacterial screening with the crude extracts [Table 4]. All fractions did not give a well-defined response against all bacteria and showed variable zones of inhibition.

From our study, it was revealed that the solvent fractions did not show a satisfactory response against tested bacterial strains at 25 mg/ml concentration. Of all the fractions, only DF was reported to be active in showing inhibitory activity against six bacteria, and the highest activity was shown against *S. pyogenes* giving the zone of inhibition of 18.67 ± 0.58 mm. The least activity was shown by AF-II against *S. pyogenes* giving the smallest zone of inhibition of 5.67 ± 0.58 mm. On the other hand, PF and AF-I of set-PCE did not inhibit the growth of any of the test bacterial isolates. The most sensitive bacterium was *S. pyogenes* showing sensitivity against six solvent fractions with considerable inhibition zones. No zones of inhibition were observed against other bacteria, *viz*., *E. faecalis*, *K. pneumoniae*, *S. typhimurium,* and *S. aureus* showing their resistance against the tested fractions. Earlier study reported *in vitro* agar diffusion sensitivity tests of crude extract fractions of the *A. nilotica* using ethanol, chloroform, methanol, petroleum ether, water, and ethyl acetate against nine bacterial isolates. All the leaves extract fractions exhibited

Table 4: Antibacterial activity of solvent fractions of *A. nilotica*

Bacterial cultures	Solvent fraction (25 mg/ml)					
	CF	EF	HF	DF	BF	AF-II
B. subtilis	-	-	-	7.33±0.58	-	-
E. coli	-	-	-	15.50±0.50	-	-
P. aeruginosa	-	-	7.67±0.29	10.17±0.29	-	-
S. typhi	-	-	9.00±0.50	10.17±0.29	-	-
S. epidermidis	-	-	-	7.33±0.58	6.50±0.50	-
S. pyogenes	11.00±0.00	10.17±0.29	18.17±0.76	18.67±0.58	12.17±0.76	5.67±0.58

Antibacterial activity was expressed in terms of diameter of zone of growth inhibition in mm (mean±SD, *n*=3). *B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, S. typhi: Salmonella typhi, S. epidermidis: Staphylococcus epidermidis, S. pyogenes: Streptococcus pyogenes*, SD: Standard deviation, AF: Aqueous fractions, PF: Petroleum spirit fraction, CF: Chloroform fraction, EF: Ethyl acetate fraction, HF: Hexane fraction, DF: Dichloromethane fraction, BF: Butanol fraction

weak or no antibacterial activity on the bacterial isolates tested. However, the stem bark and root extracts exhibited strong antibacterial activities against *K. pneumoniae*, *P. aeruginosa*, *P. vulgaris*, *S. typhi*, *S. dysenteriae*, *S. aureus,* and *E. coli* except on *S. pneumoniae* and *S. pyogenes*.[28]

In this study, the crude solvent extracts consistently displayed better antibacterial activity as compared to the solvent fractions prepared in organic solvents. The extracts and fractions unable to inhibit the growth of the bacteria showed that they may require at high concentrations, or the bacteria were resistant to these extracts. The Gram-positive bacterial strains were more susceptible to the extracts, as compared to Gram-negative bacteria. Earlier findings also reported better antibacterial activity of *A. nilotica* extracts against Gram-positive cocci than Gram-negative bacilli.[11,29] This might be because of the difference in cell wall composition of Gram-negative and Gram-positive bacteria. It has been reported earlier that the mesh-like peptidoglycan layer of Gram-positive bacteria is more accessible to permeation by the plant-origin antimicrobials as compared to Gram-negative bacteria. The presence of a thin lipopolysaccharide exterior membrane in Gram-negative bacteria acts as an effective permeability barrier which may restrict the penetration of the plant extract.[30-32]

Phytochemical Screening

All the hot and cold crude extracts were analyzed for the presence of secondary metabolites by specific reactions and identified by observing the intensity of color developed and/or the appearance of precipitation in the reactions. The secondary metabolites tested were found to be present in different crude extracts with varied intensity and concentration showing positive reactions. The compounds, which were fairly present, were symbolized as (++), slightly present as (+), whereas negative reactions (−) represent the absence of those particular compounds in respective extracts. The preliminary screening revealed the presence of cardiac glycosides, flavonoids, saponins, and tannins, whereas alkaloids and terpenoids were reported negative in all the crude extracts [Table 5].

Determination of total phenolic content

The total phenol contents of the methanolic crude extract and solvent fractions were reported as mg/g gallic acid equivalents (GAE). The standard curve of gallic acid is represented in Figure 1. The total phenol content of the methanolic crude extract was 28.44 mg/g of GAE. Among the solvent fractions, BF contained the highest (44.97 mg/g) amount of phenol compounds followed by EF (35.88 mg/g), DF (12.74 mg/g), AF-I (11.92 mg/g), PF (11.09 mg/g), CF (07.78 mg/g), AF-II (06.13 mg/g), and HF (05.30 mg/g).

Determination of total flavonoid content

The calibration curve of quercetin standard is shown in Figure 2. The total flavonoid contents in the methanolic crude extract and solvent fractions were expressed in mg

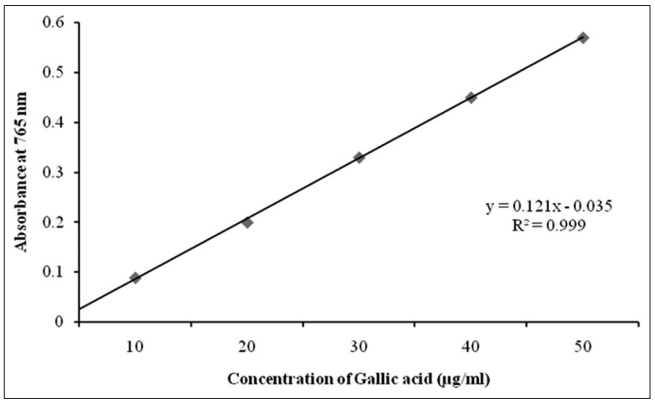

Figure 1: Calibration curve of different concentrations (µg/ml) of gallic acid and their respective optical density at 765 nm

Table 5: Qualitative phytochemical analysis of crude extracts of *A. nilotica*

Phytochemical	Tests	Butanolic		Ethanolic		Methanolic	
		Cold[a]	Hot[b]	Cold[a]	Hot[b]	Cold[a]	Hot[b]
Alkaloids	Mayer's test	–	–	–	–	–	–
	Dragendorff's test	–	–	–	–	–	–
Anthraquinones	Borntrager's test	+	++	–	–	–	+
Cardiac glycosides	Legal test	+	++	+	+	+	++
	Keller-Killiani test	+	+	+	+	+	+
Flavonoids	Alkaline reagent test	+	+	+	+	+	+
	Shinoda test	+	+	+	+	++	++
Saponins	Froth test	+	+	+	+	+	+
Tannins	$FeCl_3$ test	++	+	+	+	++	++
	Lead acetate test	++	+	+	+	++	++
Terpenoids	Salkowski's test	–	–	–	–	–	–

[a]Infusion (cold extraction), [b]Soxhlet (hot percolation), +: Positive (slightly present), ++: Positive (fairly present), –: Negative (absent)

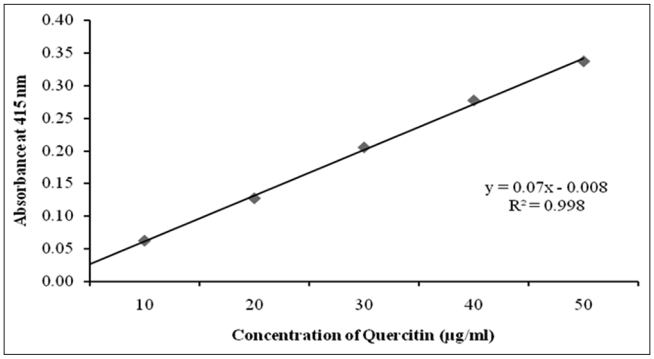

Figure 2: Calibration curve of different concentrations (µg/ml) of quercetin and their respective optical density at 415 nm

quercetin/g weight. Among all the fractions, PF contained the highest (61.50 mg/g) amount of flavonoids content followed by BF (30.08 mg/g), AF-I (28.65 mg/g), CF (24.36 mg/g), DF (17.22 mg/g), EF (17.20 mg/g), HF (08.65 mg/g), and AF-II (02.93 mg/g). Furthermore, the methanolic crude extract was reported to contain 21.50 mg/g amount of flavonoids content.

The result showed varying concentrations of total phenol and total flavonoid content in the methanolic crude extract and solvent fractions of *A. nilotica*. Taken as a whole, the BF and PF were reported to contain the highest amount of total phenol and total flavonoid content, respectively. Earlier study reported that family Mimosaceae is the richest source of phenolics (*A. nilotica*: 80.63 mg GAEs, *Acacia catechu* 78.12 mg GAEs, and *Albizia lebbeck* 66.23 mg GAEs).[33]

One of the most commonly applied procedures for the determination of total flavonoid content in medicinal plants is based on aluminum-complex formation which is measured spectrophotometrically. Flavonoids are considered as one of the most important plant secondary metabolites and are responsible for numerous biological activities.[34] Similarly, plants with a rich source of total phenols can be a promising candidate for a natural antibacterial agent. The spectrophotometric *in vitro* assay of total phenol is done by measuring the absorbance of blue color which is developed on the reaction of Folin-Ciocalteu reagent with reducing compounds including polyphenols.[35]

In this study, although the high amount of total phenol and total flavonoid content was found in some solvent fractions, this result cannot be correlated with the antibacterial activity of solvent fractions. This may be because the presence of secondary metabolites and their biological activities are highly dependent on the solvent medium used. Moreover, the expression "total flavonoid" content is not adequate as the results are dependent on the structure of the individual flavonoids present.[36] Therefore, this can be assumed that the procedure used in this study might be specific for certain compounds belonging to different classes of phenolics and flavonoids.

CONCLUSION

The overall results revealed the therapeutic use of *A. nilotica* leaves in the traditional system of medicine. The antibacterial activities of crude extracts and different fractions could be largely due to the independent or the cumulative effect of the phytochemicals detected. Although, the data showed that the antibacterial activity of *A. nilotica* may not necessary be attributed to their total phenolic and total flavonoid concentrations. Further exploration of the active constituents is necessary to determine the full therapeutic potential and possible drug development.

ACKNOWLEDGMENT

The authors are grateful to People's University, Bhopal, Madhya Pradesh, India, for providing research facilities in carrying out this project. The authors are also thankful to Sarvajanik Jankalyan Parmarthik Niyas, Bhopal, Madhya Pradesh, India, for providing this research grant.

REFERENCES

1. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. Afr J Biotechnol 2008;7:1797-806.
2. Maslin BR, Miller JT, Seigler DS. Overview of the generic status of *Acacia* (Leguminosae: Mimosoideae). Aust Syst Bot 2003;16:1-18.
3. Malviya S, Rawat S, Kharia A, Verma M. Medicinal attributes of *Acacia nilotica* Linn. - A comprehensive review on ethnopharmacological claims. Int J Pharm Life Sci 2011;2:830-7.
4. Bargali K, Bargali SS. *Acacia nilotica*: A multipurpose leguminous plant. Nat Sci 2009;7:11-9.
5. Husain SZ, Malik RN, Javaid M, Bibi S. Ethonobotanical properties and uses of medicinal plants of Morgah Biodiversity Park, Rawalpindi. Pak J Bot 2008;40:1897-911.
6. Shah SR, Hassan G, Rehman A, Ahmed I. Ethanobotanical studies of the flora of district Musakel and Barkhan in Balochistan, Pakistan. Pak J Weed Sci Res 2006;12:199-211.
7. Kubmarawa D, Ajoku GA, Enwerem NM, Okorie DA. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afr J Biotechnol 2007;6:1690-6.
8. Saini ML, Saini R, Roy S, Kumar A. Comparative pharmacognostical and antimicrobial studies of *Acacia* species (*Mimosaceae*). J Med Plants Res 2008;2:378-86.
9. Pareek A, Trivedi PC. Ethnobotanical studies on medicinal plants of Kaladera region of Jaipur district. Indian J Fundam Appl Life Sci 2011;1:59-63.
10. Sahu SC, Pattnaik SK, Sahoo SL, Lenka SS, Dhal NK.

Ethnobotanical study of medicinal plants in the coastal districts of Odisha. Curr Bot 2011;2:17-20.
11. Sharma AK, Kumar A, Yadav SK, Rahal A. Studies on antimicrobial and immunomodulatory effects of hot aqueous extract of *Acacia nilotica* L. Leaves against common veterinary pathogens. Vet Med Int 2014;2014:747042.
12. Mahmood A, Mahmood A, Qureshi RA. Antimicrobial activities of three species of family mimosaceae. Pak J Pharm Sci 2012;25:203-6.
13. Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali SM, *et al.* Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules 2009;14:586-97.
14. Sawant RS, Godghate AG, Sankpal SA, Walaki SA, Kankanwadi SS. Phytochemical analysis of bark of *Acacia nilotica*. Asian J Plant Sci Res 2014;4:22-4.
15. Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu A, Ibrahim A. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (thorn mimosa). Vet Res Forum 2014;5:95-100.
16. Hemamalini G, Jithesh P, Nirmala P. Phytochemical analysis of leaf extract of plant *Acacia nilotica* by GCMS method. Adv Biol Res 2013;7:141-4.
17. Deshpande SN. Preliminary phytochemical analysis and *in vitro* investigation of antibacterial activity of *Acacia nilotica* against clinical isolates. J Pharmacogn Phytochem 2013;1:23-7.
18. Vijayasanthi M, Kannan V, Venkataswamy R, Doss A. Evaluation of the antibacterial potential of various solvent extracts of *Acacia nilotica* linn. leaves. Hygeia J D Med 2012;4:91-6.
19. Kavitha PA, Pawan K, Narasimah Murthy TP, Gopinath SM. Methanolic extract of *Acacia nilotica* and antibacterial activity against hospital isolates of Bengaluru district. Int J Latest Res Sci Technol 2013;2:522-4.
20. Srivastava S. Understanding Bacteria. Berlin: Springer Science & Business Media; 2013.
21. Thakur R, Singh R, Jain N. Evaluation of antibacterial activity of *Sphaeranthus indicus* L. leaves. J Pharm Res 2012;5:4382-8.
22. Harbone JB. Phytochemical Method: A Guide to Modern Techniques of Plant Analysis. London: Champan and Hall; 1998.
23. Trease EG, Evan WC. Textbook of Pharmacognosy. 12th ed. Oxford: Alden Press; 2007.
24. Sultana M, Verma PK, Raina R, Prawez S, Dar MA. Quantitative analysis of total phenolic, flavonoids and tannin contents in acetone and n-hexane extracts of *Ageratum conyzoides*. Int J ChemTech Res 2012;4:996-9.
25. Yousuf M, Aslam K, Wani BA, Aslam N, Dar NA, Nawchoo IA. *In vitro* antibacterial activity and phytochemical studies of methanolic extract of leaves of *Hypericum perforatum* L. Growing wild in Kashmir Himalaya. Asian J Plant Sci Res 2012;2:414-20.
26. Rahman A, Shakoor A, Zaib G, Mumtaz AS, Ihtesham Y, Napar AA. Comparative antimicrobial activity of *Acacia nilotica* L. Leaves extracts against pathogenic bacteria and fungi. J Med Plants Res 2014;8:975-82.
27. Venkataswamy R, Doss A, Mubarack HM, Sukumar M. Phytochemical, HPTLC finger printing and antibacterial activity of *Acacia nilotica* (L.) Delile. Hygeia J D Med 2010;2:38-42.
28. Okoro SO, Kawo AH, Arzai AH. Phytochemical screening, antibacterial and toxicological activities of *Acacia nilotica* extracts. Bayero J Pure Appl Sci 2014;7:105-15.
29. Mustafa NK, Tanira MO, Dar FK, Nsanze H. Antimicrobial activity of *Acacia nilotica* sub spp. *Nilotica* fruit extracts. Pharm Pharmacol Commun 1999;5:583-6.
30. Kumar A, Rahal A, Dwivedi SK, Gupta MK. Prevalence and antibiotic profile of bacterial isolates from bovine mastitis in Mathura. Egypt J Dairy Sci 2010;38:31-4.
31. Tajkarimia MM, Ibrahima SA, Cliverb DO. Antimicrobial herb and spice compounds in food. Food Control 2010;21:1199-218.
32. Biswas B, Rogers K, McLaughlin F, Daniels D, Yadav A. Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.). On two gram-negative and gram-positive bacteria. Int J Microbiol 2013;2013:746165.
33. Sulaiman CT, Balachandran I. Total phenolics and total flavonoids in selected Indian medicinal plants. Indian J Pharm Sci 2012;74:258-60.
34. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, *et al*. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop J Pharm Res 2008;7:1019-24.
35. Mongkolsilp S, Pongbupakit I, Sae-Lee N, Sitthithaworn W. Radical scavenging activity and total phenolic content of medicinal plants used in primary health care. J Pharm Sci 2004;9:32-5.
36. Pekal A, Pyrzynska K. Evaluation of aluminium complexation reaction for flavonoid content assay. Food Anal Methods 2014;7:1776-82.

Source of Support: Nil. **Conflict of Interest:** None declared.