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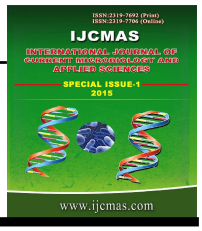

Original Research Article

Antibacterial Activity of *Acacia arabica* (Bark) Extract against selected Multi Drug Resistant Pathogenic Bacteria

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Keywords

Antibacterial activity, multi drug resistance, extracts, bioactive compounds, *Acacia arabica*

A B S T R A C T

The present study was aimed to investigate antimicrobial activity of *Acacia arabica* bark extracts against selected multi drug resistant Gram positive and Gram negative bacterial pathogens. Extracts of *Acacia arabica* bark were prepared by using hexane, petroleum ether, chloroform, ethyl acetate, acetone and methanol on the basis of their increasing polarity and were screened for the antibacterial activity by agar well diffusion assay. Acetone extract was found to be most potent against all the selected bacterial pathogens followed by methanol, chloroform, ethyl acetate and hexane while petroleum ether extract was found least effective among all. Acetone extract was further subjected to thin layer chromatography and column chromatography for the isolation and purification of bioactive compounds; thus a total of 10 fractions were obtained and were studied for their antibacterial activity through spot assay technique. Phytochemical analysis showed the presence of tannins, carbohydrates, terpenoids, phenols, anthraquinone, cardiac glycosides, flavonoids and alkaloids.

Introduction

Infectious diseases represent an important cause of morbidity and mortality among the general population, particularly in developing countries. The ability of microorganisms to acquire and transmit resistance against antibiotics causes nosocomial and community acquired infections (Mattana *et al*., 2012). The development of resistance in microorganisms to presently available antibiotics has necessitated the search for new antimicrobial agents (Negi and Dave, 2010). In developing countries about 80% of the population utilizes medicinal plants for the treatment of infectious diseases (Kim *et al*., 1987).

Indian gum Arabic tree *Acacia*, belong to the family leguminosae, and has been recognized worldwide as a multipurpose tree. *Acacia arabica* bark has been used as demulcent, nutritive supplement,

expectorant, styptic and tonic and have astringent, immunosuppressant, antibacterial, antitumor, antithrombotic, hypoglycemic and anti-helminthic activities (Rajvaidhya *et al*., 2012).

Phytochemical screening of the stem bark of *Acacia arabica* revealed that the plant contain amines and alkaloids (dimethyl tryptamine, 5-methoxy-dimethyltryptamine, and N-methyltryptamine), cyanogenic glycosides, cyclitols, saponins, fatty acids and seed oils, fluoroacetate, gums, non-protein amino acids, terpenes, hydrolyzable tannins, flavonoids and condensed tannins (Seigler, 2003). Flavonoids, sterols/triterpenoids, alkaloids and phenolics are known to be bioactive antidiabetic principles (Yasir *et al*., 2010). The bark is also reported to contain (+) catechin, (-) epicatechin, (+) dicatechin, quercetin, gallic acid, (+) leucocyanidin gallate, sucrose and (+) catechin-5-gallate (Sundaram and Mitra, 2007). *Acacia* gum contains chiefly arabin which is the mixture of calcium, magnesium and potassium salts of arabic acid. On hydrolysis arabic acid yields L-rhamnopyranose, galactopyranose, L-arabofuranose and taldobionic acid 6-d-glucuronosido-d-galactose. Further hydrolysis yields L-arabinose, D-galactose, d-glucuronic acid and rhamnose. The gum also possesses enzymes like oxidases, peroxidases and pectinases (Rajvaidhya *et al*., 2014).

Previous studies on *Acacia arabica* (Bark) showed that the plant possess antibacterial activities against various organisms. Acetone, methanol and petroleum ether extracts showed antibacterial activity against *S. aureus, S. mutans, S. sanguis, S. salivarius, L. acidophilus* and *C. albicans* (Ajaybhan *et al*., 2010). Activities were also found against *P. aeruginosa, E. coli, B. licheniformis, S.aureus, Salmonella sp., Enterobacter sp., E. coli, P. intermedia* and *P. gingivalis* (Bhatnagar *et al*., 2013). *Acacia arabica* seeds were reported to be active against *S. aureus, S. epidermidis, P. aeruginosa, K. pneumoniae, C. albicans* and *A. niger* (Parmar *et al*., 2010).

The present investigation was thus undertaken to explore the antimicrobial activity of the plant against multidrug resistant bacterial pathogens.

Materials and Methods

Collection of plant material

Barks of *Acacia arabica* collected from Allahabad were identified and authenticated through Raw Material Herbarium and Museum, CSIR-NISCAIR, New Delhi.

Test bacteria

Several Gram positive (*Bacillus cereus*, *Bacillus subtilis*, *Clostridium perfringens*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Listeria monocytogenes*) and Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Vibrio cholerae* and *Campylobacter jejuni*) bacteria with multi-drug resistance property and were previously isolated from clinical specimens were used in the present study to evaluate the antibacterial properties of *Acacia arabica* (bark) extracts.

Preparation of *Acacia arabica* (Bark) extract

The plant extracts were prepared as per the methods explained by Mattana *et al*. (2012). *Acacia arabica* bark powder was weighed in Erlenmeyer flasks of 250ml capacity. To this hexane was added and extraction process performed with constant percolation for 24–48h at 150 rpm. Then the extract was decanted and the solvent was allowed to

evaporate. This was successively extracted with petroleum ether, chloroform, ethyl acetate, acetone, and methanol. The extracts thus obtained were stored in airtight screw capped vials at -10°C until used.

Antibacterial activity of *Acacia arabica* (Bark) extract

Antibacterial activity of *Acacia arabica* bark extract was tested by using Agar well diffusion technique on Muller Hinton agar media (Agarry *et al*., 2005). The presence of zone of inhibition (mm) was indicated as the presence of antibacterial activity. Each extract of *Acacia arabica* bark was tested against the test organisms in triplicates along with media control and organism control plates.

Isolation of Antibacterial Agents

The extracts showing the antibacterial activity were selected and used for the isolation of antibacterial agent. The screening for the number of major and minor compounds present in the extract was done by thin layer chromatography (TLC). Further, the selection of solvent combination for mobile phase required to isolate the antibacterial agent through column chromatography was also decided on the basis of TLC. The compounds were isolated and purified through silica gel column chromatography.

Evaluation of antibacterial activity of different compounds

The fractions obtained from column chromatography were allowed to stand at room temperature till the entire solvent present in the compounds evaporated. Then the dried fraction was dissolved in Di-Methyl Sulfoxide (DMSO) and used for screening antibacterial activity against the selected pathogenic bacteria using Spot Assay Technique (Jack *et al*., 1995).

Phytochemical analysis

The fractions with antibacterial activity were subjected to the standard procedures of phytochemical screening for the identification of its various active constituents (Tannins, Anthraquinone, Saponin, Cardiac glycoside, Flavonoids, Reducing sugars, Catechol, Alkaloids, Terpenoids, Phenol, Carbohydrate test) (Trease and Evans, 1989; Sofowora, 1993).

Statistical analysis

The effect of various extracts obtained from individual *Acacia arabica* (Bark) extract was analysed using TWO Way classification analysis of variance (ANOVA), and F-test at 5% and 1% significance level (Fisher and Yates, 1990).

Result and Discussion

Antibacterial activity

The extracts prepared from *Acacia arabica* bark using different solvents showed varying degree of antimicrobial activity against both Gram positive and Gram negative multi drug resistant organisms selected for the study. Among the extracts prepared using different solvents, acetone extract was found to be effective against all the organisms except *C. jejuni*, followed by methanol, chloroform, ethyl acetate and hexane while petroleum ether was found least effective ($P<0.001$). All the test organisms were found to be sensitive to all the extracts prepared in the study. Maximum activity with respect to zone of inhibition was recorded for *E. coli* (18.67–31.00 mm) followed by *S. pyogenes* (11.00–29.33 mm), *B. cereus* (12.67–27.67 mm), *V. cholerae* (17.33–24.67 mm), *S. aureus* (17.67–22.67 mm), *B. subtilis* (16.00–27.67 mm), *P. aeruginosa* (13.00–32.00 mm), *S.*

dysenteriae (12.67–24.33 mm), *C. perfringens* (15.00–27.33 mm), *S. typhi* (11.00–26.00 mm), *L. monocytogenes* (14.00–21.67 mm), while *C. jejuni* was found to be least sensitive with no activity recorded with acetone extract ($P<0.001$) (Table 1).

Isolation of different compounds in the *Acacia arabica* (Bark) Acetone extract

On the basis of the performance of antimicrobial activity, Acetone extract was used for further investigation. Using solvents in combinations of 2 and 3, 24 compounds were detected by TLC (Table 2).

A total of 282 fractions were collected from column chromatography which was finally pooled into 10 fractions on the basis of R_f values in respective solvent systems (Table 3).

Antibacterial activity of different fractions obtained in column chromatography

Fractions pooled after column chromatography were evaluated for their antibacterial activity against the test pathogens. Low to medium activity was demonstrated by all the fractions against the selected pathogens with fraction number 4 and 7 exhibiting maximum activity against *S. aureus* and *S. dysenteriae,* respectively (Table 4).

Qualitative phytochemical screening of the *Acacia arabica* (Bark) extracts

All the extracts of *Acacia arabica* (Bark) were screened for the presence of various phytochemical components *i.e.* Tannins, carbohydrates, catechol, terpenoids, phenol, anthraquinone, saponins, cardiac glycosides, flavonoids, alkaloids and reducing sugars. The extracts were found to be positive for a number of bioactive compounds (Table 5).

In the present era where medicinal plants are becoming the most preferable source for the isolation of some new bioactive chemical compounds, the plant used in the present study proved as a good source of efficient bioactive compounds in inhibiting broad category of multi drug resistant bacteria. This was supported by some of the earlier studies done to determine the antibacterial activity of *Acacia arabica* (Bark) against bacterial pathogens *viz*., *S. typhi, S. aureus, B. subtilis, E. coli, S. epidermidis, P. aeruginosa, S. viridians, S. sonnei* and *P. fluroscence* (Mbatchou *et al*., 2011; Malviya *et al*., 2011; Rajvaidhya *et al*., 2012; Bhatnagar *et al*., 2013).

In the study acetone and methanol extract of *A. arabica* bark was found to be most effective in exhibiting antibacterial activity. Literature survey has revealed few studies that are comparable with the present findings. Patel *et al*. (2009) reported maximum antibacterial activity of methanolic extract of *A. arabica* bark followed by chloroform and least with petroleum ether extract against *S. aureus, P. aeruginosa* and *E. coli*. Sharma *et al*. (2014) evaluated antibacterial activity of *A. arabica* bark extracts in different solvents against *S. aureus, P. aeruginosa* and *E. coli* and observed maximum activity with acetone followed by methanolic extracts. Other workers have also reported extracts made in methanol to be most potent against *S. aureus, P. aeruginosa, B. subtilis, E. coli* (Deen and Sadiq, 2002; Kavitha *et al*., 2013) and *S. typhi* (Mbatchou *et al*., 2011). However some variations have been observed in studies quoted in the literature.

Table.1 Antibacterial activity of *Acacia arabica* (Bark) extracts against selected Gram positive and Gram negative bacteria

S.No.	Organisms	Zone of Inhibition (mm diameter)					
		Hexane	Pet. Ether	Chloroform	Ethyl Acetate	Acetone	Methanol
1	*Listeria monocytogenes*	18.00 ± 3.00	15.67 ± 2.08	18.67 ± 3.06	21.67 ± 2.08	14.00 ± 1.00	18.00 ± 3.00
2	*Bacillus cereus*	20.00 ± 2.00	12.67 ± 2.08	22.33 ± 4.04	23.33 ± 4.16	27.67 ± 2.52	22.00 ± 2.00
3	*Bacillus subtilis*	16.67 ± 0.58	16.00	16.67 ± 0.58	20.33 ± 0.58	27.67 ± 0.58	20.00
4	*Clostridium perfringens*	15.33± 0.58	15.00 ± 1.73	17.00 ± 1.00	18.00 ± 1.00	27.33 ± 2.52	18.67 ± 1.53
5	*Staphylococcus aureus*	17.67± 2.08	19.00 ± 2.00	22.67 ± 1.53	22.00 ± 2.65	21.33 ± 1.53	22.00 ± 3.00
6	*Streptococcus pyogenes*	18.00 ± 2.00	21.67 ± 2.08	21.67 ± 0.58	11.00 ± 1.00	29.33 ± 0.58	26.67 ± 0.58
7	*Escherichia coli*	18.67 ± 2.08	13.67 ± 3.79	21.33 ± 1.53	23.00 ± 5.29	31.00 ± 1.00	24.00 ± 2.65
8	*Campylobacter jejuni*	15.57 ± 0.58	16.33 ± 0.58	17.33 ± 0.58	16.33 ± 0.58	0.00	16.33 ± 0.58
9	*Vibrio cholerae*	18.33 ± 1.15	17.33 ± 2.31	20.33 ± 3.21	21.67 ± 4.73	24.67 ± 5.03	22.33 ± 3.21
10	*Pseudomonas aeruginosa*	16.00 ± 1.00	13.00 ± 2.65	16.00 ± 1.00	21.33 ± 1.53	32.00 ± 2.65	17.00 ± 2.00
11	*Shigella dysenteriae*	20.00 ± 1.00	24.33 ± 3.06	22.67 ± 2.08	12.67 ± 0.58	13.00 ± 1.00	21.67 ± 1.53
12	*Salmonella typhi*	15.00 ± 5.29	14.67 ± 3.51	20.33 ± 1.53	11.00 ± 1.00	26.00 ± 1.00	22.33 ± 3.21

Table 2 Compounds detected in the *Acacia arabica* bark acetone extract

S. No.	Solvent Combination	R. F Value
1.	Acetone: Hexane 4:6	0.137, 0.224
2.	Acetone: Hexane 6:4	0.548
3.	Acetone: Petroleum Ether 2:8	0.072
4.	Acetone: Petroleum Ether 3:7	0.233
5.	Acetone: Petroleum Ether 4:6	0.228
6.	Acetone: Petroleum Ether 6:4	0.5, 0.75, 0.8, 0.866
7.	Acetone: Chloroform 4:6	0.703
8.	Acetone: Chloroform 6:4	0.857
9.	Acetone: Petroleum Ether: Chloroform 5:1:4	0.50877
10.	Acetone: Petroleum Ether: Chloroform 6:2:2	0.693
11.	Acetone: Petroleum Ether: Chloroform 7:2:1	0.745, 0.847
12.	Acetone: Chloroform: hexane 8:1:1	0.532
13.	Acetone: hexane: petroleum ether 1:2:7	0.615
14.	Acetone: hexane: petroleum ether 2:3:5	0.633
15.	Acetone: hexane: petroleum ether 4:1:5	0.241
16.	Acetone: hexane: petroleum ether 5:2:3	0.277
17.	Acetone: hexane: petroleum ether 7:2:1	0.576, 0.7288
18.	Acetone: hexane: petroleum ether 8:1:1	0.859

Table.3 Fraction with different solvent combination isolated in column chromatography

Fraction No.	Solvent system (mobile phase)	R_f Value	Fraction No.
1.	Acetone : Hexane (4:6)	0 .78 and 0.88	1 to 32
2.	Acetone : Hexane (6:4)	1.00	33 to 72
3.	Acetone : Petroleum ether (2:8)	0.50	73 to 96
4.	Acetone : Petroleum ether (3:7)	0.60	97 to116
5.	Acetone : Petroleum ether (4:6)	0.75	117 to 144
6.	Acetone : Petroleum ether (6:4)	0.90	145 to 184
7.	Acetone : Chloroform (6:4)	1.00	185 to 215
8.	Acetone: Petroleum ether : Chloroform (7:2:1)	1.00	216 to 247
9.	Acetone : Chloroform : Hexane (8:1:1)	1.00	248 to 267
10.	Acetone : Hexane : Petroleum ether (8:1:1)	1.00	268 to 282

Table.4 Antibacterial fractions isolated from acetone extract of *Acacia arabica*

Organism	**Antibacterial activity**		
	+	++	+++
Listeria monocytogenes	1-6,8,10	7,9	-
Bacillus cereus	1,6	2-5,7-9	-
Bacillus subtilis	1,6,8-10	2-5,7	-
Clostridium perfringenes	1,2,8	4-7,9,10	-
Staphylococcus aureus	1,3,6	2,5,7-9	4
Streptococcus pyogenes	1,3,4,6,8,10	2,5,7,9	-
Escherichia coli	4,5,8	1,3,7,9,10	-
Campylobacter jejuni	1-3,6	4,5,7-10	-
Vibrio cholera	1,3-6,8,10	2,7,9	-
Pseudomonas aeruginosa	1-6,8-10	7	-
Shigella dysenteriae	1,2,5,6,8	3,4,9,10	7
Salmonella typhi	1-8,10	9	-

+ Low; ++ medium: +++High

Table.5 Qualitative phytochemical screening of the *Acacia arabica* (Bark) extracts

Test	Extract					
	Hexane	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol
Tannins	–	–	–	+	+	+
Carbohydrates	–	–	–	+	+	+
Catechol	–	–	–	–	–	–
Terpenoids	+	+	+	+	+	+
Phenol	–	–	–	+	+	+
Anthraquinone	–	–	–	+	+	+
Saponin	–	–	–	–	–	–
Cardiac glycosides	–	–	+	+	+	+
Flavonoids	–	–	–	+	+	+
Alkaloids	–	–	–	–	–	–
Reducing sugars	–	–	–	–	–	–

In the study conducted by Nagumanthri *et al*. (2012) no antimicrobial activity was observed in the methanolic bark extract of *A. nilotica* against the test bacteria with the exception of *B. circulans*. Okoro *et al.* (2014) reported ethanol and chloroform / water extracts of *A. nilotica* stem bark to have highest antibacterial activity against the test organisms followed by methanol and ethyl acetate. The activity was tested against nine bacterial isolates *viz., K. pneumonia, E. coli, P. aeruginosa, P. vulgaris, S. typhi, S. dysenteriae, S. aureus, S. pneumonia*e and *S. pyogenes*.

The differences may be attributed to the fact that effectiveness of the extracts largely depends on the kind of solvent used. Further, the concentration of the extract and kind of bacteria may also account for the difference in the susceptibility pattern of the test organism.

In addition, as suggested by some workers (Nikaido and Vaara, 1985; Priya and Ganjewala, 2007), variation in the rate of penetration of active ingredients in the cell wall and cell membrane of the test organisms could be responsible for differences in the susceptibility pattern.

Variation in R_f values of phytochemicals provides a very important clue in understanding of their polarity and helps in selection of appropriate solvent system for separation of pure compounds by column chromatography (Talukdar *et al*., 2010). The successful separation of active constituents by chromatographic technique depends upon suitable solvent system which needs an ideal range of partition coefficient for each target compound. Further, number of fractions eluted is dependent on various factors viz., extraction method, solvent system and elution process adopted (Rajvaidhya *et al*., 2014).

The antimicrobial potency of plants has been attributed to their secondary metabolites. The present study revealed presence of tannins, carbohydrates, terpenoids, phenol, anthraquinone, cardiac glycosides and flavonoids in the ethyl acetate, acetone and methanol extracts of *A. arabica* bark.

Similar observations have been reported in studies cited in the literature with some variations (Banso, 2009; Prabhat *et al*., 2010; Mbatchou *et al*., 2011; Jacknoon *et al*., 2012; Shakya *et al*., 2012; Biswas and Roymon, 2013; Godghate *et al*., 2014). Acharyya *et al*. (2009) reported that presence of polyphenols and flavonoids in extracts are related to bactericidal activity.

In the present era where microorganisms are acquiring and transmitting resistance towards antibiotics, the rich diversity of plants can be explored for screening and evaluation for their antimicrobial activity which may provide new bioactive substances. The results of the study strongly advocate further investigation into pharmacological properties of secondary metabolites of higher plants.

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