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IN-VITRO ANTIMICROBIAL EFFECT OF *ADHATODA VASICA, ANNONA SQUAMOSA, ALOE VERA, BUTEA MONOSPERMA* AND *PROSOPIS JULIFLORA*

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ABSTRACT

In-vitro antibacterial activity of aqueous, alcoholic, and chloroform extracts of leaves of *Adhatoda vasica*, *Annona squamosa*, *Butea monosperma*, *Prosopis juliflora* and *Aloe vera* leaf pulp against various animal bacterial isolates was investigated by disc diffusion assay. Different extracts were also screened qualitatively for the presence of active phytochemicals and total extractability was also calculated. Amongst different plant extracts *P. juliflora* methanolic extract has shown good antibacterial activity against *B. cereus*, *E. coli*, *P. vulgaris*, *S. aureus* and *S. agalactiae* except *S. typhimurium.* Methanolic extract of the plant has shown highest mean zone of inhibition (mm) 23.13 ± 0.34 against *S. agalactiae.* This extract was also significantly effective against *B. cereus.* Methanolic extracts of *A. squamosa* and *B. monosperma* were effective *against E. coli* with mean zone of inhibition (mm) 22.73 ± 1.17 and 19.70 ± 0.87 respectively. *A. vasica* water extract has shown little activity against *S. agalactiae.* Phytochemical analysis has revealed variation in presence of alkaloids, triterpenes, tannins, steroids, flavonoids, glycosides, saponins, and carbohydrates in different extracts of plants. Methanolic extract of *Prosopis juliflora* has shown presence of alkaloid that might have resulted in antimicrobial effect of plant which should be further explored for its *in-vivo* efficacy in animal infections.

KEYWORDS: Antimicrobial activity, Phytochemical analysis, *Adhatoda vasica*, *Annona squamosa*, *Butea monosperma*, *Prosopis juliflora*, *Aloe vera*

INTRODUCTION

Medicinal plants are important natural antimicrobial resources that can be used in controlling the infections in human and animals.[1] Isolation and validation of active antibacterial compounds from medicinal plants permit the synthesis of newer drug. It is beneficial to explore the medicinal properties of plants collected from different sources. Active compounds such as alkaloids, glycosides, volatile oils, gums, tannins, steroids, saponins, phlobatannins, flavonoids and other secondary metabolites present in the plants might be responsible for the antibacterial action against microorganisms.[2]

Adhatoda vasica (Family Acanthaceae) is a shrub commonly known as "Ardusi or Vasaka". It is origin of Asian continent and used in respiratory disorders like bronchitis, asthma and whooping cough. *Annona squamosa* L. (Family Annonaceae) known as "Custard apple" is cultivated in various parts of India. Its leaves are used in the treatment of maggot wound and dermatitis in animals.[3] *Aloe vera* (*Aloe barbadensis* Mill.) (Family Liliaceae) possesses anti-bacterial, anti-fungal and antioxidant effect.[4] *Butea monosperma* L. (Family Fabaceae), is traditionally used for the treatment of inflammatory diseases. Its leaves possess antimicrobial property and anthelmintic activities [5] *Prosopis juliflora* (Family Fabaceae) is commonly known as "Mesquite". It is a fast growing, thorny deciduous, drought-resistant plant and has a wide crown and deep-rooted. The crude extracts of various parts of *Prosopis juliflora* and its purified chemical components have been found to possess antimicrobial, insecticidal and different pharmacological activities.[6]

The information on *in-vitro* antimicrobial activity of aqueous, methanol and chloroform extracts of leaves of *Adhatoda vasica*, *Annona squamosa*, *Butea monosperma*, *Prosopis juliflora* and *Aloe vera* leaf pulp against gram-positive and gram-negative bacterial isolates from animals like *B. cereus*, *E. coli*, *P. vulgaris*, *S. aureus*, *S. agalactiae*, *S. typhimurium* are not available. Thus, the present study was carried out to evaluate *in-vitro* antimicrobial activity of various crude extracts of these plants and the presence of active phytochemical constituents.

MATERIALS AND METHODS

Plant material and extracts preparation

The leaves of *A. vasica, A. squamosa, A. vera, B. monosperma* and *P. juliflora* were collected from premises of Junagadh Agricultural University Campus, Junagadh and authenticated by Botanist. The collected leaves were washed thoroughly with running tap water and finally with sterile distilled water. *A. vasica, A. squamosa, B. monosperma* and *P. juliflora* powders were made after shade drying and used to prepare different solvent extracts (water, methanol and chloroform) by using soxhlet extraction unit.[7] *A. vera* leaves were washed with distilled water and fresh pulp was collected. The pulp was dried in an oven at 60^0C for 48 hours and then powdered. Aqueous, methanol and chloroform extracts were obtained by soaking 25 grams of the powder in 200 ml of each solvent for 48 hours then contents were filtered through Whatman filter paper no.1 and concentrated by evaporation.[8] All extracts were concentrated under reduced pressure with rotary vacuum evaporator. The concentrated extracts were transferred to previously weighed petri dish and allowed to evaporate till they free from the solvent. The yield in percentage was estimated by weighing the petri dish again. The percent extractability of plants with respect to the powdered material was calculated as total amount of extract obtained X 100/ total weight of powder taken for extraction. The dried extracts were labeled as crude extracts and stored at -20^{o}C for further use.

Chemicals and reagents

All chemical of analytical grades were procured from Merck Pvt. Ltd. or SD fine Pvt. Ltd., India. Nutrient broth, Nutrient agar, MRS agar, MRS broth, TSA agar, TSA broth, Muller Hinton Agar, Sterile blank disc and antibacterial discs were procured from Himedia Lab, India. Different types of media were prepared for sub-culturing and to test antimicrobial activity of plant extracts. Standard antibacterial discs [tetracycline (30 mcg), gentamicin (10 mcg), levofloxacin (05 mcg), ceftriaxone (30 mcg), penicillin-G (10 unit) and ampicillin (10 mcg)] were also used to observe the sensitivity of bacteria.

Preliminary phytochemical analysis

Presences of active phytochemical constituents in different extracts of plants were identified by various chemical test methods.[9, 10]

Test Micro Organisms

Animal isolate cultures of *Bacillus cereus, Escherichia coli, Proteus vulgaris Staphylococcus aureus, Streptococcus agalactiae* and *Salmonella typhimurium* were procured from

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Preparation of antimicrobial disc

Dried water extracts were reconstituted with sterile distilled water where as methanol and chloroform extracts were reconstituted with dimethyl sulphoxide (DMSO- 2% solution) at concentration of 200 mg/ml. Solution of extracts (50 µl) were dispensed on blank sterile discs and sterilized under UV light for 24 h and stored in refrigerated conditions until used in disc diffusion assay.

Determination of antibacterial activity

Antibacterial activity of different extracts was evaluated using Muller Hinton agar (MHA) plate by disc diffusion assay.[11] The entire antibacterial assay was carried out under strict sterile conditions. Turbidity of broth was corrected by adding sterile saline until 0.5 McFarland turbidity standard of 10^6 Colony Forming Unit (CFU) per ml was achieved. Standard antibacterial discs like tetracycline, gentamicin, levofloxacin, ceftriaxone, penicillin-G and ampicillin were used to compare the zone of inhibition mm of plant extracts against various gram positive and gram-negative bacteria. Data were analyzed by one way ANOVA followed by Duncan Multiple Range Test.

RESULTS

The percent extractability and phytochemical constituents of different extracts of plants are depicted in table 1 and table 2, respectively. Antibacterial activity was evaluated by inhibition of bacterial growth surrounding the plant extract discs and by measuring zone of inhibition mm with digital vernier caliper (Mitutoyo, Japan). The assay was carried out in triplicates. Mean ± S.E. zone of inhibition (mm) of different extracts of *A. vasica, A. squamosa, A. vera, B. monosperma P. juliflora* against bacterial isolates was given in table-3 and graphically presented in figure 1. Mean ± S.E. zone of inhibition (mm) of different antibacterials against bacterial isolates was given in table-4 and graphically presented in figure 2. Zone of inhibition (mm) of different extracts *P. juliflora* against *S. aureus* and *S. agalactiae* were depicted in figure 3 and 4 respectively.

Table 1: Physical characteristics and percent extractability of the different extracts of *A. vasica, A. squamosa, A. vera, B. monosperma* and *P. juliflora.*

Name of Plant	Physical characteristics			Per cent extractability		
	WE	ME	CE	WE	ME	CE
A. vasica	Dark brown, Solid	Green, Semi-solid, Viscous	Black, Solid , Viscous	7.92	19.44	4.88
A. squamosa	Dark brown, Oily, Semi-solid,	Dark green, Semi solid, Creamy	Green, Solid Viscous	33.10	33.50	7.24
A. vera	Brown Oily,	Dark green, Semi solid	Green, Semi solid	5.67	7.35	3.59
B. monosperma	Brown, Solid	Black, Semi-solid , Viscous	Green, Solid	7.52	15.24	3.88
P. juliflora	Brownish, Semi solid, Pasty	Black, Semi-solid , Crystalloid	Dark green, Solid	14.76	42.80	10.68

WE= Water extract, ME=Methanolic extract, CE=Chloroform extract

Table 2: Phytochemical constituents of different extracts of *A. vasica, A. squamosa, A. vera, B. monosperma* and *P. juliflora.*

Phyto-Chemicals	A. *vasica*			*A. squamosa*			*A. vera*			*B. monosperma*			*P. juliflora*		
	WE	ME	CE	WE	ME	CE	WE	ME	CE	WE	ME	CE	WE	ME	CE
Steroid	-	+	-	-	-	+	+	-	-	-	-	+	-	+	-
Triterpene	-	+	-	-	-	+	-	+	+	-	+	-	-	+	-
Alkaloid	-	+	+	-	-	+	-	+	+	-	-	-	+	+	+
Tannin	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-
Flavonoid	-	+	+	-	+	+	-	+	-	-	-	+	+	-	-
Glycoside	-	-	-	-	-	-	-	+	-	-	-	+	-	+	-
Saponin	-	+	-	-	-	-	+	+	-	-	-	-	+	+	-
CHO	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-

WE= Water extract, ME=Methanolic extract, CE=Chloroform extract, CHO: Carbohydrate; +/- = Presence/absence of phytochemical constituents

Table 3: Zone of inhibition mm (Mean ± S.E.) of different extracts of *A. vasica, A. squamosa, A. vera, B. monosperma P. juliflora* against bacterial isolates.

Plant extract	*B. cereus*	*E. coli*	*P. vulgaris*	*S. typhimurium*	*S. aureus*	*S. agalactiae*
A. vasica (W)	----	----	----	----	----	10.73 ± 0.85^{a}
A. vasica (M)	----	----	----	----	----	----
A. vasica (C)	----	----	----	----	----	----
A. squamosa (W)	12.53 ± 0.48^{ab}	14.40 ± 0.46^{ab}	----	----	----	14.37 ± 0.96^{ab}
A. squamosa (M)	----	22.73 ± 1.17^{b}	----	----	----	----
A. squamosa (C)	----	18.70 ± 1.04^{b}	----	----	----	----
A. vera (W)	----	----	16.80 ± 0.83^{a}	----	----	----
A. vera (M)	13.57 ± 0.58^{abc}	14.27 ± 0.64^{ab}	----	9.65±0.72	12.03 ± 0.95^{a}	20.50 ± 0.67^{bc}
A. vera (C)	12.10 ± 0.25^{ab}	8.93 ± 0.20^{a}	----	----	13.43 ± 0.62^{a}	----

B. monosperma (W)	----	19.67±0.22^{b}	----	----	----	----
B. monosperma (M)	16.37±0.81^{c}	19.70±0.87^{b}	----	----	----	----
B. monosperma (C)	11.50±0.55^{a}	----	----	----	----	----
P. juliflora (W)	12.90±0.91^{abc}	----	----	----	17.70±0.62^{ab}	16.20±0.20^{abc}
P. juliflora (M)	15.33±0.38^{bc}	21.07±0.83^{b}	14.17±0.35^{a}	----	20.60±0.38^{b}	23.13±0.34^{c}
P. juliflora (C)	----	22.23±0.96^{b}	15.80±0.52^{a}	----	17.63±0.28^{ab}	20.37±0.69^{bc}

Different superscript in a column indicates significant difference between mean (P<0.05)

Table 4: Zone of inhibition in mm (Mean ± S.E.) of different antibacterials against bacterial isolates.

Antibacterials	**Bacterial isolates**					
	B. cereus	***E. coli***	***P. vulgaris***	***S. typhimurium***	***S. aureus***	***S. agalactiae***
Tetracycline	25.37±0.43^{b}	30.83±0.55^{b}	26.63±1.13^{b}	21.13±0.87^{ab}	34.47±0.55^{b}	34.13±0.56^{c}
Gentamicin	24.23±0.72^{ab}	30.27±0.67^{b}	13.30±0.49^{a}	17.83±0.55^{ab}	25.40±0.46^{ab}	23.20±0.53^{ab}
Levofloxacin	23.47±0.54^{ab}	39.10±1.08^{b}	22.20±0.70^{ab}	29.80±0.90^{b}	36.53±1.13^{b}	32.10±0.76^{bc}
Ceftriaxone	22.73±0.78^{ab}	31.30±0.70^{b}	25.43±1.42^{b}	28.03±0.43^{b}	30.13±0.37^{ab}	22.40±0.50^{a}
Penicillin-G	15.80±0.92^{a}	----	13.07±0.52^{a}	10.63±1.15^{a}	18.38±0.74^{a}	29.37±0.48^{abc}
Ampicillin	31.30±0.66^{b}	23.80±0.35^{b}	16.27±0.93^{ab}	23.93±1.23^{b}	24.77±0.88^{ab}	35.00±0.44^{c}

Different superscript in a column indicates significant difference between mean (P<0.05)

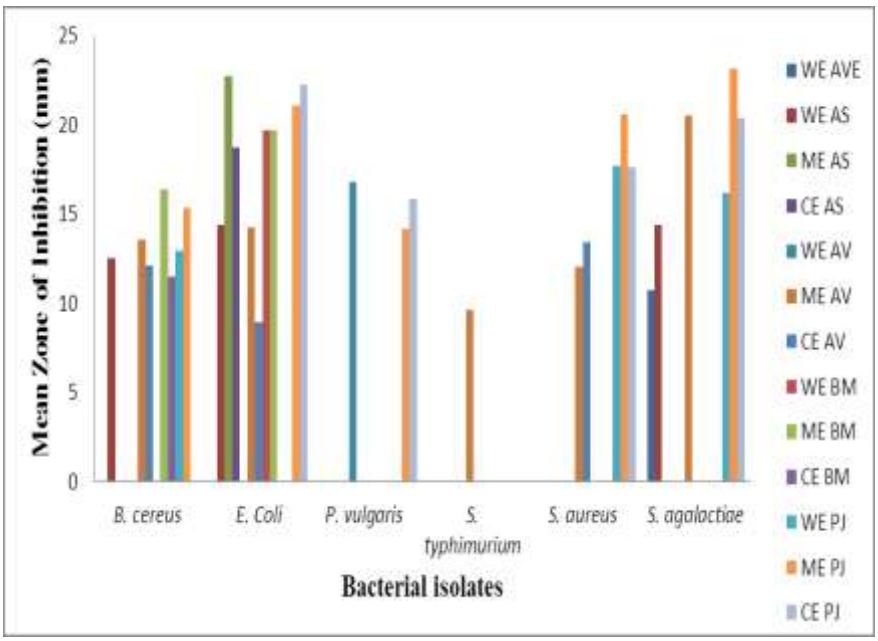

Figure 1: Zone of inhibition mm (Mean ± S.E.) of different extracts of *A. vasica, A. squamosa, A. vera, B. monosperma P. juliflora* against bacterial isolates.

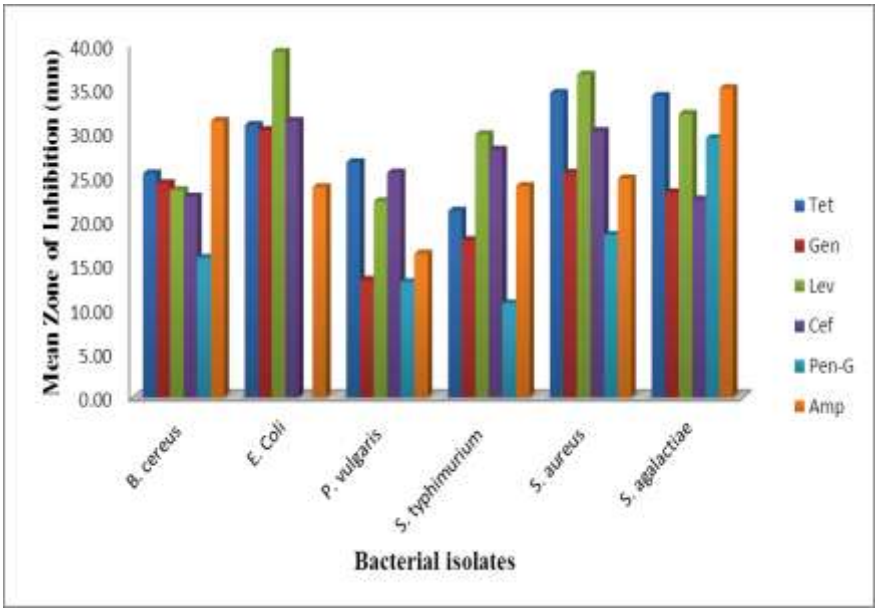

Figure 2: Zone of inhibition mm (Mean ± S.E.) of different antibacterials against bacterial isolates.

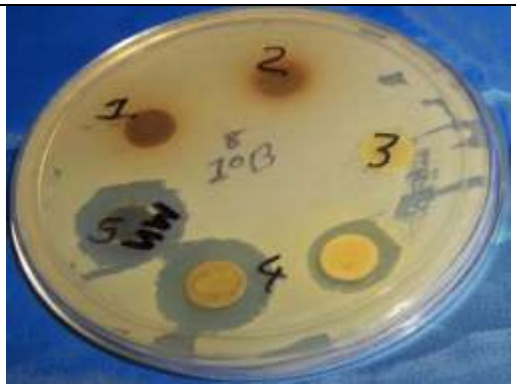

Figure 3: Zone of inhibition (mm) of Different extracts of *P. juliflora* against *S. aureus*

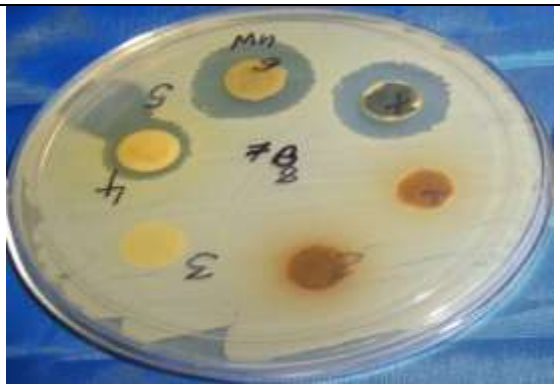

Figure 4: Zone of inhibition (mm) of Different extracts of *P. juliflora* against *S. agalactiae*

DISCUSSION

In the present study, *Aloe vera* methanolic extract was found with lower values of zone of inhibition (mm) against *B. cereus*, *E. coli*, *S. typhimurium*, *S. aureus* and *S. agalactiae* that is lower than earlier reported antibacterial effect of *Aloe vera* methanolic extract with zone of inhibition against *E. coli* (25.00±0.00), *B. cereus* (18.00± 0.00) and *S. aureus* (15.50±0.00).[12] Similar higher activity of *Aloe vera* methanolic extract against *S. aureus* (16.60 ±1.53) and *E. Coli* (15.60 ± 1.53) was also reported.[13] *A. squamosa* water extract was found effective against *B. cereus* (12.53 ±0.48), *E. coli* (14.40±0.46) *and S. agalactiae* (14.37±0.96). Water extract *of A. vasica* also shown activity against *S. agalactiae* with mean zone of inhibition of 10.73 ± 0.85 mm.

B. monosperma methanolic extract has shown good zone of inhibition (mm) against *B. cereus* (16.37±0.81) and *E. coli* (19.70±0.87) which was higher than the previously reported activity against *E. coli* (12.00±0.00) [5] but lower than the ethanolic extract activity of plant against *E. Coli* (28.63 ± 0.64).[14]

P. juliflora methanolic extract has shown antibacterial activity against all bacteria except *S. typhimurium. P. juliflora* methanolic extract has shown highest mean zone of inhibition in mm against *S. agalactiae* (23.13 ± 0.34) is similar to that of ceftriaxone (22.40 ± 0.50). Methanolic extract has also shown significant higher effect as compare to other extract against *B. cereus* (15.33±0.38), *E. coli* (21.07±0.83), *P. vulgaris* (14.17±0.35) and *S. aureus* (20.60±0.38), which was comparable or higher than reported values against *E. coli* (15.33 ± 0.58), *S. typhi* (07.00 ± 0.00) and *S. aureus* (08.00 ± 0.00).[15] Some researchers have also reported lower antibacterial activity of methanolic extract of *P. juliflora* than present study against *E. coli*, *S. aureus*, *B. cereus* and *Salmonella sp.* with zone of inhibition (mm) of 12.81±0.45, 12.72±0.67, 13.12±0.37 and 11.04±0.33 respectively.[16] *P. juliflora* chloroform extract is also having good antibacterial effect with zone of inhibition in mm against *E. coli* (22.23±0.96), *P. vulgaris* (15.80±0.52), *S. aureus* (17.63±0.28) *and S. agalactiae* (20.37±0.69).

Among different antibacterials, ceftriaxone has shown good *in-vitro* activity against *B. cereus E. coli*, *P. vulgaris*, *S. typhimurium*, *S. aureus* and *S. agalactiae* with zone of inhibition of 22.73±0.78, 31.30±0.70, 25.43±1.42, 28.03±0.43, 30.13±0.37 and 22.40±0.50 mm, respectively. *E. coli* is equally susceptible to all antibacterial drug except penicillin-G. Tetracycline has shown significantly higher effect 34.47±0.55 than ceftriaxone 30.13±0.37 against *S. aureus*. Tetracycline and ampicillin has shown good *in vitro* activity against *S. agalactiae*. The results of the study indicated that the methanolic extract was more effective than aqueous extract. This may be due to the higher content of active principle having antibacterial activity with better solubility in organic solvents.

CONCLUSION

In conclusion, methanolic extract of *Prosopis juliflora* shown good *in-vitro* antibacterial activity against several gram-positive and gram-negative bacteria which may be due to presence of active phytochemical ingredients like alkaloids and it should be further explored for its *in-vivo* efficacy in animal infections. *Anona squamosa* and *Butea monosperma* extracts were also found effective against gram-negative bacteria. *Aloe vera* extracts shown

comparatively less anti-bacterial activity. Isolation and identification of active compounds from different crude extracts must be explored to evaluate its potential as antimicrobial activity.

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