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THE PHYTOCHEMICALS FROM THE TRADITIONAL MEDICINAL PLANT, *ADHATODA VASICA* AS POTENT ANTIBACTERIAL AGENT AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been emerging as a cardinal thereat worldwide in communities and hospitals. The bacterium readily acquires resistance against all classes of antibiotics due to either mutation of an existing bacterial gene or horizontal transfer of a resistance gene from another bacterium. This has prompted a desperate search for novel chemotherapeutics to treat acute infections from MRSA. In this investigation, antibacterial compounds from the roots of the Ayurveda Medicinal plant, *Adhatoda vasica* were isolated by soxhlet extraction. The different extracts were tested for their inhibitory activity against methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 25923 strains by the disc diffusion method. The residue obtained from the dichloromethane extract showed significant zone of inhibition of 18mm. This antibacterial activity was comparable to that shown by Linezolid and, Vancomycin with zones of inhibition values 20 and 19 mm respectively. This points to the promising future of the phytochemicals present in the dichloromethane extract individually or in synergy as effective antibacterial agent(s) against MRSA.

Keywords: Phytochemicals, Methicillin-resistant *Staphylococcus aureus,* Antibacterials, *Adhatoda vasica*

INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen asymptomatically transmitted to the human body. Methicillin-resistant strains of *S. aureus* (MRSA) includes those strains with the gene giving them resistance to methicillin and all other beta-lactam antibiotics due to the selective pressure of the current regime of antibiotics **[1, 2]**. Now, it has emerged as pathogenic and leading pathogen causing high rate of patient morbidity and mortality. The multi drug genotype possessed by MRSA has lead to its resistance to even the glycopeptides **[3, 4]**. However, as a result of limited tissue distribution, as well as the emergence of isolates with reduced susceptibility and *in vitro* resistance, there is an urgent need for the development of alternative therapeutic that target MRSA. MRSA is especially troublesome in hospitals where patients with open wounds, invasive devices and weakened immune systems are at greater risk of infection than the general public.

Phytochemicals are upsurging as newer and safer therapeutics with potential as antibacterials **[5]**. The recent researches focused on natural products have shown a useful way to obtain a potentially rich source of drug candidates, where alkaloids and flavanoids have been found more effective **[6]**. One of the ways to prevent antibiotic resistance of pathogenic species is to use new lead compounds that are not based on existing synthetic antimicrobial agents. In this stance, it is imperative to look into the chances of the compounds obtained from plants for their pharmacological assay in view of the vast source of innumerable therapeutic effects representing molecular diversity engineered by nature **[7]**.

The recent research reports the efficacies of various plant extracts and other phytochemicals against the increasing incidence of MRSA **[8, 9]**. Herein, we report the anti-MRSA activity shown by the dichloromethane extract of the roots of an Ayurvedic medicinal plant, *Adhatoda vasica* and the antibacterial activities of the extracts were compared with antibiotics of known potencies. The present work points to the futuristic applications of the phytochemicals as anti-infective agents.

MATERIALS

The plant, *Adathoda vasica* was collected from Pathanamthitta District, Kerala, India in the month of December. It was authenticated by the Taxonomist of Amrutha Arya Drugs, Koodal, Pathanamthitta, Kerala, India who are the GMP certified producers and expprters of Ayurvedic drugs. The root of the plant was

separated and dried at 40^0C and employed for further extraction after pulverization. The photograph of the plant is given in **Figure 1**.

Figure 1: *Adhatoda vasica* Plant collected

CHEMICALS

The chemicals, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), *L*-ascorbic acid and N,N-dimethylsulphoxide, procured from Merck, were used as such. The HM agar and nutrient broth employed for the antibacterial activity studies were purchased from Himedia, India. . Unless otherwise specified, all the other reagents were of analytical reagent grade. Solvents employed were either of 99% purity or purified by known laboratory procedures **[10]**.

METHODS

Extraction

The Soxhlet extraction method was employed for the pulverized root using a sequential method with solvents in the increasing polarity as hexane, dichloromethane, ethyl acetate and methanol. The residue was obtained by solvent removal or evaporation under reduced pressure.

Analytical Methods

Phytochemical Analysis of the Various Extracts

The phytochemical analyses of the hexane, dichloromethane and ethylacetate extracts of A. *vasica* root for alkaloids, saponins, tannins, glycosides,anthraquinones, terpenes, and flavonoids were carried out by reported procedures **[11, 13]**.

Antioxidant Activity Studies

The Free radical scavenging activity ofthe residues were evaluated by the DPPH method **[14]**.

Anti Bacterial Activity Studies

Preparation of Inoculum

The identified bacterial isolates were cultured on separate nutrient agar plates and incubated at 37^oC for 48 hrs and checked for the appearance of colonies. A loopful of isolated colonies were inoculated into 4 ml of peptone water, incubated at 37°C for 24 hrs. This actively growing bacterial suspension was then adjusted with peptone water so as to obtain a turbidity visually comparable to that of 0.5 McFarland standard prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride

dihydrate ($BaCl_2$. $2H_2O$) with 99.5 ml of 1% (v/v) sulphuric acid (H_2SO_4). This turbidity is equivalent to approximately $1–2 \times 10^8$ colony forming units per ml (CFU/ml).

Evaluation of Antibacterial Activity of Extracts by Well Diffusion Method

Agar diffusion method was employed to evaluate the antibacterial activity. *S. aureus* ATCC 25923 isolates were tried in this study. Wells of 8mm diameter was incised on agar. The bacteria growth was swabbed on Mueller Hinton agar and 100µl of the residue was added into wells. After incubation at 37°C for 24 hrs, diameter of zone of inhibition was measured and consequently antibacterial activity was assessed. The average value of diameter of zone of inhibition exhibited by sensitive isolates to antibiotics and extracts were recorded. All the experiments were conducted in triplicates and an average value was taken. A comparative study was carried out by disc diffusion against antibiotics of known potencies.

UV-VIS Spectral Analysis

The UV-Vis spectrum of the residues was recorded in a Systronics 2022 Spectrophotometer.

RESULTS AND DISCUSSION

The results of the chemical analyses of the various extracts are prearranged in **Table 1**. The hexane extract was devoid of tannins due to the inherent high polarity of the poly phenolic groups present. The saponins are secondary metabolites those are amphipathic glycosides and hence these were present in the highly non polar hexane and polar ethylacetate. The chemical analyses proved that amino acids are totally absent in the roots of *A. vasica.* All the extracts showed characteristic absorption bands at λ_{max} values 220, 264 and 285nm. Three characteristic absorptions in the UV region between 210-290 nms were reported as characteristic to alkaloids derived from anthranilic acid. The Acanthaceae is one among the plant families with a rich source of quinozoline alkaloids which are derived from anthranilic acid. *Adhatoda* belongs to this family.

The free radical scavenging activity of each extract was measured and represented graphically in **Figure 2**. DPPH model was employed in the evaluation of anti oxidant activities of the residues obtained from each extraction. The observed activity was compared graphically with that of vitamin C measured under identical conditions. The results are given in **Table 2**. The hexane

extract showed the lowest inhibition of 27 % at 0.20 mg/mL concentration. The dicholomethane extract has higher activity which is 89% at 0.20 mg/mL concentration but lower than that shown by the standard, vitamin C. The ethylacetate residue exhibited 78% inhibition at a concentration of 0.20 mg/mL which was well below that of the standard. The IC_{50} values of the ethylacetate and dichloromethane extracts are determined graphically and compared with that of vitamin C in **Table 3**. The IC_{50} value of the dichloromethane extract is very significant. The antibiogram of the various root extracts against *Staphylococcus aureus* ATCC 25923 is noted in **Table 4**. The anti bacterial activity can be seen in **Figure 3**. The antibiotic resistance shown by this bacterial strain is given in **Table 5**. It is evident that the above strain has multiple antibiotic resistance. The reference standard for the systemic infection by MRSA, vancomycin and limited exposure antibiotic Linezolid were the only antibiotics which are effective. It was resistant to all other antibiotics screened in the present study. The zone of inhibition values were 20, 19 and 18mm for vancomycin, linezolid and the dichloromethane extract. This study is significant as the phytochemicals have spare adverse effects and are cost effective.

Table1: Phytochemical Analysis of *Adhatodavasica* Root Extract

TYPE OF EXTRACT	PHENOLS	TANNINS	ALKALOI-DS	SAPONI-NS	FLAVA-NOIDS	REDU-CING SUGAR	AMINO ACIDS	STEROIDS
Hexane	+	-	+	+	+	+	-	+
Ethyl acetate	+	+	+	+	+	+	-	+
Dichloro methane	+	+	+	-	+	+	-	+

+ Presence of phytochemical compounds; - Absence of phytochemical compounds

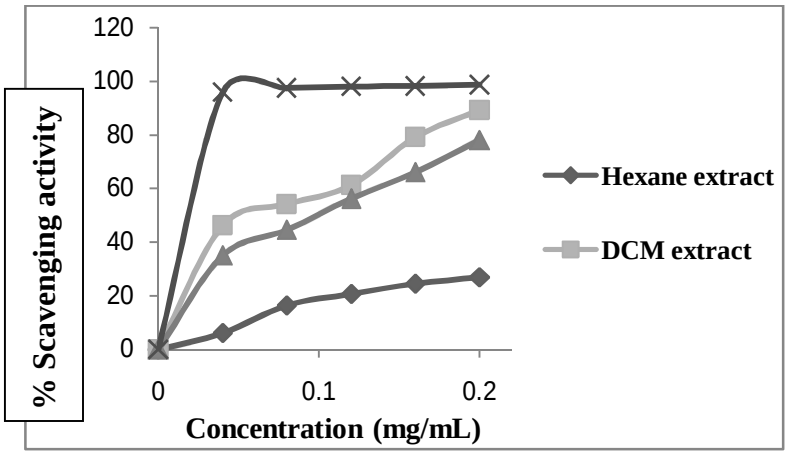

Figure 2: Free Radical Scavenging Activity of the *A.vasica* Root Extracts and Vitamin C

Table 2: Free Radical Scavenging Activity of the Various Root Extracts of *A. vasica* and Vitamin C

Conc. (mg/mL)	% Inhibition by			
	Hexane extract	DCM extract	Ethylacetate ectract	Vitamin C
0.04	6.032	46.23	35.021	96.024
0.08	16.458	54.17	44.552	97.486
0.12	20.634	61.22	56.149	98.067
0.16	24.517	79.12	66.138	98.214
0.20	26.942	89.16	78.064	98.673

Table 3: IC_{50} Values

S. No.	COMPOUND	IC_{50} value (mg/mL)
1.	DICHLOROMETHANE EXTRACT	0.045
2.	ETHYLACETATE EXTRACT	0.090
3.	VITAMIN C	0.012

Table 4: Antibiogram of Root Extracts

S. No.	EXTRACT	ZONE OF INHIBITION AGAINST *S. AUREUS* ATCC 25923
1.	HEXANE	-
2.	DICHLOROMETHANE	18
3.	ETHYLACETATE	-

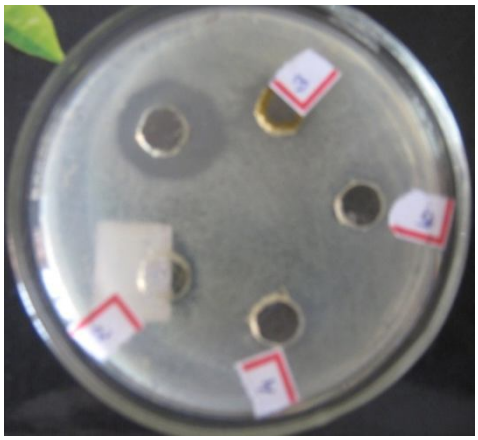

Figure 3: Antibacterial Activity Against Standard Strain of *S. aureus ATCC 25923*

Table 5: Antibiotic Resistance by *S. aureus*, ATCC 25923

S. No.	Name of the antibiotic	Code and potency(mcg)	Diameter of zone of inhibition (mm)	Result
1	Cefoxitin	Cn- 30	-	R
2	Oxacillin	Ox- 1	-	R
3	Penicillin,	P- 10 units	-	R
4	Cephalexin,	Cp/Cn-30	-	R
5	Cotrimoxazole,	Co- 25	13	R
6	Amoxycillinclavulanic acid	Ac—30 (20+10)		R
7	Gentamycin	G-10	-	R
8	Linezolid	Lz-30	20	S
9	Vancomycin,	Va 30	19	S

CONCLUSIONS

The present study conclusively proved the efficacy of the phytochemicals present in the dichloromethane extract of the roots of the Ayurvedic medicinal plant *A. vasica* against a multi drug resistant strain of *S. aureus***,** ATCC 25923. The zone of inhibition obtained was comparable to that of the potent antibiotics, vancomycin and linezolid. The future of this study is the identification of the individual components present in the extract and a synergistic study with these components together or in combination with the antibiotics also. In this way the gene pools of the multi drug resistance can be effectively reduced in near future.

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