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IN-VITRO ANTIBACTERIAL SCREENING OF THE EXTRACTS OF SWERTIA CHIRAYITA LINN. AGAINST MRSA (METHICILLIN RESISTANT *Staphylococcus aureus*)

Abdul Latif¹, Sumbul Rehman¹, Shamim Ahmad², Asad U Khan³

¹Department of Ilmul Advia (Unani Pharmacology & Pharmaceutical Sciences)
Faculty of Unani Medicine, Aligarh Muslim University, Aligarh

²Department of Microbiology, Institute of Ophthalmology, AMU Unit, Gandhi Eye Hospital, Aligarh

³Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh

E-mail of corresponding author: abdullatifamu@gmail.com

ABSTRACT

Aqueous and ethanolic extract of Chirayita (*Swertia chirayita* Linn.) were screened for their antibacterial activity Methicillin Resistant *Staphylococcus aureus* (MRSA). Kirby Bauer's Disk Diffusion method and Broth Serial Dilution method according to CLSI Guidelines by W.H.O were used. It was compared with the Standard drug-Vancomycin and Plane control-DMSO (the solvent used). The Resistant drug-Methicillin was also used to confirm the resistance of MRSA strains used. The prepared plates were incubated and results were evaluated by measuring the Zone of Inhibition - ZOI (in mm.) of drug extract. MIC and MBC was also carried out against the resistant strain. All the experiments were conducted in triplicates and in sterilized conditions. The results were analyzed statistically using gpaidd software and it was found that MRSA strain was sensitive to *S.chirayita* showing a significant ZOI as compared to the standard drug. Present investigation indicates that *S.chirayita* possesses antimicrobial properties and hence can be used for future natural plant based antimicrobial agents. Thus the study provides an in-vitro proof of its antibacterial activity against MRSA. However further investigations are needed to find out its pharmacological details and to make it fit for use, for the welfare of humankind. So, that it can be used against the infectious diseases caused by the resistant strain of *Staphylococcus aureus* safely and effectively.

Keywords: *Swertia chirayita* Linn. MRSA, Methicillin, Vancomycin

INTRODUCTION

Over the last three decades Methicillin Resistant *Staphylococcus aureus* is responsible for causing several difficult-to-treat infections in humans. Also known as Multidrug resistant *S.aureus* or Oxacillin resistant *S.aureus* (ORSA) emerged as a noscomial pathogen in early 1960s [1] it is resistant to a large group of antibiotics called

beta-lactams, which include the penicillin and the cephalosporins. A study led by the CDC (Centre for Disease Control) and published in the October 17, 2007 issue of the Journal of the American Medical Association estimated that MRSA would have been responsible for 94,360 serious infections and associated with 18,650 hospital stay-related deaths in the United States in 2005. These figures suggest that MRSA infections are responsible for more deaths in the U.S. each year than AIDS. In 2007, the CDC reported that MRSA causes 19,000 deaths every

year in the US, which is more than HIV/AIDS cases [2].

The studies done so far about the increasing incidence of MRSA infection are motivating the researchers to find out new pharmacological components of 'natural origin'. Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms [3]. The worldwide emergence of Resistant strains of *Staphylococcus aureus* has become a major therapeutic problem. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity [4]. Researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against multidrug resistant microbe strains. A wide group of medicinal plant preparations are available that have been used over the centuries almost exclusively on the basis of empirical evidence [6]. The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment [7]. The World Health Organization estimates that 65%-80% of the world's population use traditional medicine as their primary form of health care. The use of herbal medicine, the dominant form of medical treatment in developing countries, has been increasing in developed countries in recent years [8].

Swertia chirayita (Family Gentianaceae) is used in traditional medicines since the period of Dioscorides. It is rich in a wide variety of secondary metabolites such as alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties [6]. Phytochemical analysis reveals that it contains a yellow bitter acid Ophelic acid ($C^{15}H^{20}O^{13}$), two bitter glucosides-chiratin ($C^{26}H^{48}O^{15}$) and amarogentin ($C^{32}H^{38}O^{16}$); gentiopicrin, two yellow crystalline phenols, xanthone-swerchirin [9-13].

It has been found to have anti-carcinogenic, antiviral, antihelminthic, antimalarial, anti-pyretic, anti-inflammatory, anti-arthritic activity [14-20]. There are several reports on the antimicrobial activity of its extracts [21]. These evidences contribute to support and quantify the importance of screening *S. chirayita*.

The pharmacological potential of *Swertia chirayita* reveals that its antibacterial activity towards a large number of bacterial strains, but towards MRSA its activity has not been done so far, so this was an attempt to screen its in-vitro antibacterial activity against the resistant bacteria.

MATERIAL AND METHODS

The herb was procured from the local market Baradari of Aligarh city and was properly identified by the Botanical literature available and then confirmed by Prof. S. H. Afaq from the Pharmacognosy section, Department of Ilmul Advia, Aligarh Muslim University, Aligarh. Voucher specimens (SC-0100/09-G) were preserved in the herbarium of Medicinal Plant Lab in the Department of Ilmul Advia, F/O Unani Medicine, Aligarh Muslim University, Aligarh for future reference.

Preparation of plant extract

Two different extract were prepared for analysis in the present study viz. aqueous extract and ethanolic extract as per W.H.O guidelines with some minor modifications [22].

For aqueous extract: 10 gm of the powdered drug and 150 ml of the Double Distilled Water (DDW) were put into a soxhlet apparatus. The solvent was boiled at 40°C and refluxed for a period of 150 min (eleven extraction cycles). The extract was filtered and evaporated to dryness under reduced pressure in the Lyophilizer (Macro Scientific works, Delhi). It was redissolved in DMSO (Dimethyl Sulphoxide) to the desired concentration (20 mg/ml) for the study.

Similarly for the **ethanolic extract:** 10 gm of the powdered drug and 150 ml of the ethanol (Solvent) were put into a soxhlet apparatus and the same procedure was repeated as stated above.

Microorganisms used

Clinical strains of MRSA isolated from various sources viz. pus (Pus culture: PC), urine (Urine Culture: UC) and control strains N315, Mu50, ORSA (Oxacillin resistant *Staphylococcus aureus*) of the tested microorganisms were obtained from Department of Microbiology, JNMC & Hospital, AMU; Microbiology Section, Institute of Ophthalmology, JNMC & Hospital, AMU, Aligarh. The bacterial cultures were grown in Nutrient Broth (M002 Himedia Labs, Mumbai, India) and incubated at 37°C for 24 hours, followed by frequent sub culturing to fresh media and were used as test bacteria. The bacterial cultures were checked to confirm the presence of sufficient number of bacterial cells on nutrient broth and maintained on nutrient agar slant.

Antimicrobial activity

Antimicrobial assay of the crude extracts was performed against pathogenic strains by Kirby Bauer's Disk diffusion method and Agar well method [23, 24]. The nutrient agar plates were swabbed with a suspension (10^6 cfu/ml) of the bacterial strains. The wells of the equivalent size were prepared with the help of a cork borer and the drug (40µl) was poured in the respective

well with the help of a micropipette. Finally, the resistant antibiotic–Methicillin disks (SD137, Himedia Labs, Mumbai, India) were placed on the prepared plates with sterile forceps and pressed properly to make complete contact with the surface of the medium. Later on these plates were kept at room temperature for 30 minutes (Pre-diffusion time) and then incubated at 37°C for 24 hours.

The antibiotic disks (6 mm) were used as Positive Control while the solvent used for diluting the test drug was used as the Negative Control. The diameters of the inhibition zone – Zone of Inhibition (ZOI) in mm was measured and is given in Table-1. The experiment was done in triplicate and the mean values were calculated.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of various extracts against tested microorganisms was determined by broth dilution method [25]. 96-well microtitre plates were used, 50 µl of standardized suspension of a strain (10^6 cfu/ml) (cfu-colonies forming unit) was added to each tube containing extracts at various concentrations. The plates were incubated at 37°C for 24h and observed for visible growth. The minimum inhibitory concentration (MIC) is taken as the lowest concentration of the extracts at which there is turbidity after incubation, the values are given in Table-2. Minimum Bactericidal Concentration (MBC) was further determined from the same isolates MBC is the minimal concentration of drug needed to kill most (99.9%) of the viable organisms after incubation for 24 hours.

STATISTICAL ANALYSIS OF DATA

All the values have been expressed as Mean ± SEM (Standard error of mean). Statistical significance was determined by one way ANOVA (Analysis of variance) using g-paid software for calculation.

RESULT

The aqueous and ethanolic extracts of *S.chirayita* exhibit varying degree of inhibitory effect against all tested pathogenic strains (Table-1). The MIC of crude extracts of the drug was determined at the concentrations ranging from 19.53 to 5000 µgm/ml (Table-2).

The aqueous extract produced zone of Inhibition in the range of 8.8 to 12.0 mm for all the tested strains while ethanolic extract produced comparably larger zone against them in the range of 23 to 30 mm, the results were found to be significant with $p\text{-value}>0.0001$. The minimum inhibitory concentration varies in the range of 625 to 1250 µgm/ml for the aqueous extract while it was lesser towards ethanolic extract in the range of 78.12 to 312.5 µgm/ml of the *S.chirayita* extract.

Aqueous extract of the test drug sample was found to have lesser activity as compared to ethanolic extract, but both extract showed larger zone as compared to the inhibitory zone of Vancomycin, which is considered as effective drug for MRSA at present. DMSO- the solvent used to dissolve the drug was also tested; it has not shown any inhibitory effect towards the tested bacteria. This confirms that the inhibitory activity of the extract was only due to the drug used and not due to the solvent in which it was dissolved. Further the resistance to Methicillin was maintained on the nutrient plate, as the antibiotic disk does not produce any inhibitory effect, this confirms the resistance of the tested strains.

DISCUSSION

Medicinal plants are naturally gifted with invaluable bioactive compounds which form the backbone of traditional medicines. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind [26]. In the present

investigation, *in vitro* antimicrobial efficacy of the crude extracts of *S.chirayita* was quantitatively assessed on the basis of inhibition zone and minimum inhibitory concentration. The study shows that the crude ethanolic extract of *S.chirayita* showed more pronounced antimicrobial activity as compared to aqueous extracts, and when efficacy of either extract was compared to Standard drug 'Vancomycin' it was found to be quite efficacious showing a greater ZOI.

The result of the present investigation suggests that *S.chirayita* is a potent natural source of biologically active compounds from herbal medicines, which may potentially prove to be efficient natural antimicrobial agents. This antibacterial property of *S.chirayita* can be attributed to the presence of the biologically active constituents present in it like amarogentin, swerchirin, triterpenoids, xanthenes, ophelic acid, gentiopicrin [27]. This study also supports the previous antibacterial activity of the chirayita extract towards gram positive bacterial strains [28] and verifies the claims of traditional medicine for their use in various infectious diseases [29-31]. However exactly which constituent is helping in killing these dreadful bacteria and in which manner, this part of the study is further need to be explored. And more investigations are needed in this direction before its use in clinics, as the present study is just an in-vitro proof about its antibacterial efficacy; many studies regarding its clinical use are still needed to be done.

CONCLUSION

With the emergence and widespread occurrence of multi drug resistant bacteria focus has now been shifted in exploring natural compounds that may combat drug resistance problem. Clearly strategic planning for search on fundamental and safe integration of efficacious medicine into conventional medical practice is needed. On the

other hand the drugs used in traditional system of medicine could be systematically explored for novel bioactive compounds which can inhibit drug resistance bacteria or which can enhance activity of antibiotics. Such compounds or herbal medicine may find application in combination with antibiotic for the treatment of bacterial diseases.

The study suggests that *S.chirayita* exhibit antimicrobial properties against MRSA which is an emerging cause of a number of infectious diseases and has developed resistance to the synthetic antibiotics. The potential antimicrobial activity of *S.chirayita* towards the infectious micro-organism explains the basis for its use in future in combating the disease caused by such dreadful bacteria.

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Graphical Presentation of Antibacterial Activity of *Swertia chirayita* Linn.

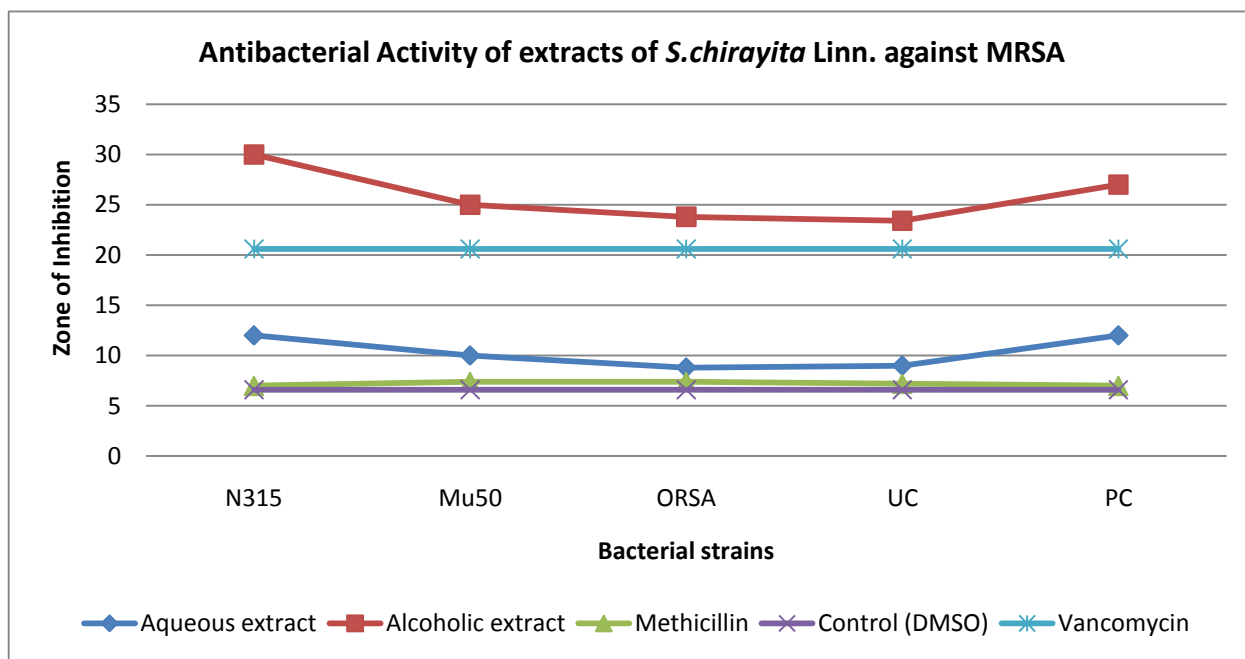


Table: 1 Zone of Inhibition (in mm) of *Swertia chirayita* Linn. Extract against MRSA strains

S.No	MRSA strains	Zone of Inhibition (ZOI) in mm (Mean ± S.E)				
		Aqueous extract	Alcoholic extract	Methicillin (30 µgm)	DMSO (50 µgm)	Vancomycin (30 µgm)
1.	N315	12 ± 0.32	30 ± 0.71	7.0 ± 0.32	6.6 ± 0.32	20.6 ± 0.32
2.	Mu50	10 ± 0.07	25 ± 0.83	7.4 ± 0.24	6.6 ± 0.32	20.6 ± 0.32
3.	ORSA	8.8 ± 0.58	23.8 ± 0.8	7.4 ± 0.24	6.4 ± 0.24	20.6 ± 0.32
4.	UC	9.0 ± 0.44	23.4 ± 0.2	7.2 ± 0.20	6.6 ± 0.32	20.6 ± 0.32
5.	PC	12 ± 0.20	27 ± 0.67	7.0 ± 0.31	6.6 ± 0.24	20.6 ± 0.32

PC: Strain isolated from a pus culture

UC: Strain isolated from urine culture

ORSA: Oxacillin Resistant Staphylococcus aureus

DMSO: Dimethyl Sulphoxide

Table-2 MIC and MBC of the extracts of *S. chirayita* Linn. against MRSA

S.No.	MRSA Strains	Aqueous extract		Alcoholic extract	
		MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
1.	N315	625	1250	78.12	312.5
2.	Mu50	1250	2500	312.5	625.0
3.	ORSA	625	2500	312.5	1250
4.	UC	625	2500	312.5	1250
5.	PC	1250	2500	625	1250