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Tephrosia purpurea–An Effective Anti-bacterial Agent Against the Clinical Isolates of Methicillin-resistant *Staphylococcus aureus*

APOORVA RANA[1], ANUSHKA CHATTERJEE[2], MANISH GOYAL[1], GAJENDRA KUMAR ASERI[3], SANDEEP K. SHRIVASTAVA[4] AND NEERAJ KHARE*

Institute of Allied Medical Science and Technology, NIMS University, Jaipur-303 121 (Rajasthan), India (e-mail: neerajsnkhare@gmail.com; Mobile: 70735 92400)

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ABSTRACT

The methicillin resistant *Staphylococcus aureus* (MRSA) is considered to be amongst the most prevalent microorganisms contributing towards the outspread of antimicrobial drug resistance. To overcome this threat, there is an urgent requirement of some effective antimicrobial agents. The present study was planned to screen the anti-MRSA potentials of the methanolic and ethanolic extracts of leaves and roots of *Tephrosia purpurea*. The ethanolic roots of *Tephrosia* were shown to possess the best anti-MRSA potentials (Activity Index = 0.56) amongst the extracts tested. The results demonstrated that *Tephrosia* extracts should be further analyzed to identify the bioactive phytoconstituents and the possible mechanism responsible for plant's anti-MRSA potentials.

Key words: MRSA, medicinal plants, *Tephrosia purpurea*, activity index

INTRODUCTION

Antibacterial drug resistance has become a major concern in all the developed and developing countries. The pool of antimicrobial agents available today has lost its effectiveness against these drug resistant microorganisms. A number of different mechanisms contribute towards the occurrence and outspread of microorganisms showing resistance against the drugs. Also, a trend is shown in the establishment of the new antibiotics and the microorganisms acquiring resistance against them within a short span of time (Pourmand *et al.*, 2017; Rosini *et al.*, 2020). The 2020 report of WHO states that antimicrobial drug resistance is amongst the top 10 threats to the universal health of humankind (WHO, 2020). Health concern is not the only problem; it is also significantly affecting the economic costs. Of all the drug resistant microorganisms, the methicillin resistant *Staphylococcus aureus* (MRSA), categorized as a "Superbug", is a vicious human pathogen giving rise to an immeasurable range of Community Acquired Infections as well as the Hospital Acquired Infections (Sunagar *et al.*, 2016; Chakraborty *et al.*, 2018). *S. aureus*, a gram positive cocci and normal micro-biota of the human skin, nose and large intestine, is amongst the most prevalent microorganisms giving rise to skin and soft tissue infections (Bassetti *et al.*, 2017; Bouvet *et al.*, 2017; Turner *et al.*, 2019). As per the Indian reports on antimicrobial resistance, methicillin resistance is observed in almost 43.6% of the *S. aureus* isolates (Gandra *et al.*, 2017). MRSA was an evident cause of hospital acquired infections (Hospital Acquired MRSA) in the 1960s, but later on in the 1990s, it progressed to be a significant cause of community acquired infections (Bassetti *et al.*, 2019; Copin *et al.*, 2019). Another type of MRSA, the Livestock Associated MRSA (LA-MRSA), has also been observed to be an evident cause of human infections (Ceballos *et al.*, 2020; Gebreyes *et al.*, 2020). The fact sheet of the healthcare associated infections, given by the

[1]Dr. B. Lal Institute of Biotechnology, Jaipur-302 071 (Rajasthan), India.
[2]Jayshree Periwal International School, Jaipur-302 017 (Rajasthan), India.
[3]Amity Institute of Microbial Technology, Amity University Rajasthan, Kant-kalwar, Jaipur-303 002 (Rajasthan), India.
[4]Centre for Innovation, Research & Development (CIRD), Dr. B. Lal Clinical Laboratory Pvt. Ltd., Jaipur-302 017 (Rajasthan), India.

WHO, states that the risk of getting the intensive care unit (ICU) infections by the low and middle income countries is approximately two to three times higher in comparison to the high income countries (WHO, 2011).

The available pool of drugs which was once effective in curing different infections is now failing to do so as a consequence of the resistance that the microorganisms have acquired against the available pool of drugs (McEwen and Collignon, 2018). To control the outspread of these resistant bacteria, there is an urgent need of efficient and quality antimicrobial agents. Efficient and effective health care systems need to be planned to escalate the success rates of treating both minor and major infections (WHO, 2020). Medicinal plants have exhibited remarkable roles in people's lives in India as well as in different ancient communities (Bhagat *et al.*, 2019, 2020; Biradar *et al.*, 2020; Ugboko *et al.*, 2020; Gadisa and Tadesse, 2021). The medicinal plants consist of a wide variety of bioactive components which contribute towards their antimicrobial potentials, amongst which the alkaloids, flavonoids, phenols and tannins are considered to be the most important (Al-Ansari *et al.*, 2019). Medicinal plants have been used in India for over 5000 years in Ayurveda as potential antifungals, antibiotics, antiparasitic, antihelminthic, analgesics, cardioprotective, antineoplastic, antihelminthic, etc. (Anand *et al.*, 2019, 2022; Tiwari *et al.* 2021a, b; Verma *et al.* 2021a, b, 2024). Many medicinal plants are shown to possess antibacterial activities against *S. aureus* as well as MRSA. In addition to this, many other medicinal plants like *Ocimum basilicum* L., *Syzygium aromaticum* L., etc., exhibit strengthening effects on many available antibiotics like penicillin, gentamycin, cephalexin and oxytetracycline, effective against *S. aureus* (Chakraborty *et al.*, 2018).

Tephrosia purpurea, commonly known by the name Wild Indigo, belongs to the Fabaceae family. In Ayurveda, it is a well-known traditional medicinal plant and given the name "*Sarwawranvishapaka*" owing to its wound healing properties. This plant has been traditionally used for treating liver disorders, asthma, impotency, kidney and spleen enlargement, etc. Different modern pharmacological studies have also demonstrated that the plant possesses antimicrobial, anticarcinogenic, antioxidant, anti-diarrheal, antispermatogenic, antifertility and hepatoprotective properties. In addition to all these properties, this plant has also been used for the development of drugs for neurological disorders like dementia and Alzheimer's (Padmapriya *et al.*, 2017; Rao *et al.*, 2020). The present research study was planned to analyze the antibacterial potentials against the MRSA clinical isolates and the phytochemicals present in the crude extracts of *T. purpurea* leaves and roots.

MATERIALS AND METHODS

Bacterial isolation was done from clinical samples like blood, urine, pus, sputum, etc. at the Microbiology Department of Dr. B. Lal Clinical Laboratory Pvt. Ltd, Jaipur, Rajasthan, India. These bacterial isolates along with the reference strains, MRSA ATCC 43300 and *S. aureus* ATCC 25923, were obtained from the Microbial Culture Repository Division catered by Centre for Innovation Research and Development, Dr. B. Lal Institute of Biotechnology. The isolated bacteria were further screened for identification as *S. aureus* and MRSA.

The isolated bacteria were processed using Gram's staining and biochemical tests: catalase and coagulase for identification of *S. aureus*. The *S. aureus* isolates were also identified by sub-culturing on Mannitol Salt Agar. The identified *S. aureus* isolates were further processed using Disk Diffusion test for the identification of MRSA isolates as a result of which a total of 38 pure MRSA isolates were obtained. These isolates along with the reference strains were stored on Nutrient Agar Slants at 4°C for further execution.

Fresh and healthy leaves and roots of *T. purpurea* were collected from Jaipur and Jodhpur. For eliminating dust and foreign particles, the gathered plant material was washed and cleansed thoroughly with water, normal saline and ethanol. The processed plant material was then shade-dried at room temperature for approximately four days. The dried material was grinded into a fine powder form.

Ten g each of the powdered leaves and roots were taken for plant extraction. The solvents, ethanol and methanol, were used in the

quantity of 200 ml for each powdered plant material and extract preparation was done using the Soxhlet extraction method, which took approximately 24 to 48 h. For the screening of phytochemicals, the concentrated extracts were used while for analyzing the antibacterial potentials, completely dried extracts were used.

The Agar Well Diffusion assay, based on the Kirby Bauer Method, was done to screen the antibacterial activity of the plant extracts against the MRSA clinical isolates (Kilany, 2016). A fresh bacterial suspension in peptone water was prepared such that its density got equivalent to the density of the 0.5 McFarland standard. Different concentrations of the plant extracts (400, 200 and 100 mg/ml) were prepared in dimethyl sulfoxide (DMSO). Pure DMSO was taken as the negative control, while streptomycin in the concentration of 5 mg/ml was used as the positive control. On the muellerhinton agar plates, wells, 6 mm in diameter, were punctured and 100 µl of bacterial suspension was then evenly distributed onto the agar surface using sterile cotton swabs. Then 50 µl of each plant extract and the controls were put into the wells and the plates were then incubated for 24 h at 37°C. After incubation, using the zone measurement scale, the diameters of the zones of inhibition (halo) were measured. The mean of zones of inhibition of each concentration and control were calculated. The activity index was calculated to analyze the antibacterial potentials of *T. purpurea* leaves and roots against MRSA clinical isolates.

$$\text{Activity index} = \frac{\text{Mean zone of inhibition of plant extract}}{\text{Mean zone of inhibition of positive control)}}$$

RESULTS AND DISCUSSION

Mannitol salt agar showed colonies of *Staphylococcus aureus*. Bubbles were observed in catalase test and coagulation was observed in coagulase test, all of which confirmed the bacterial isolates as *S. aureus* (Figs. 1, 2 and 3). The disk diffusion test performed with the *S. aureus* isolates showed the zone of inhibition of < 28 mm for penicillin and < 21 mm for cefoxitin, which confirmed them as MRSA isolates (Fig. 4).

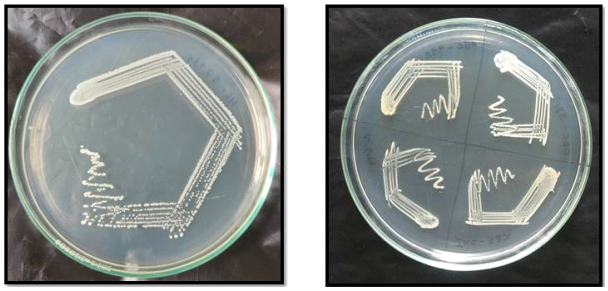

Fig. 1. Isolation of bacterial isolates from clinical samples of infected patients by streaking method.

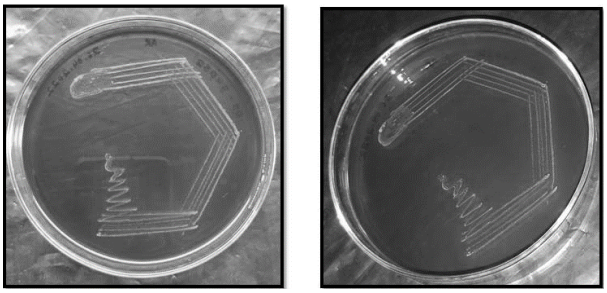

Fig. 2. Mannitol salt agar plates showing colonies of *S. aureus*.

Against 38 MRSA clinical isolates and both the reference strains (MRSA – ATCC 43300 and *S. aureus*– ATCC 25923), the antibacterial activity of the methanolic and ethanolic extracts of *T. purpurea* leaves and roots was screened. Amongst all the four extracts, the *T. purpurea* roots were shown to exhibit better antibacterial potentials against MRSA isolates. When compared amongst the solvents, ethanolic extracts of roots and methanolic extracts of leaves exhibited better anti-MRSA potentials (Table 1; Fig. 5). Overall analysis as per the activity indexes indicated that the ethanolic extracts of *T. purpurea* roots had the strongest anti-MRSA potentials amongst all the four extracts (Table 2, Fig. 6).

The antimicrobial drug resistance has become an alarming situation for the entire world. Different bacterial strains are contributing towards this alarming situation. One such microorganism is the methicillin resistant *Staphylococcus aureus* (MRSA), which is categorized as a "Superbug" by the WHO. This organism is prevalent in causing many infections in humans which makes it even more urgent to develop some promising agents that can be used to curb the problem of drug resistance. The recent research studies indicate the use of plants as promising agents to control this alarming

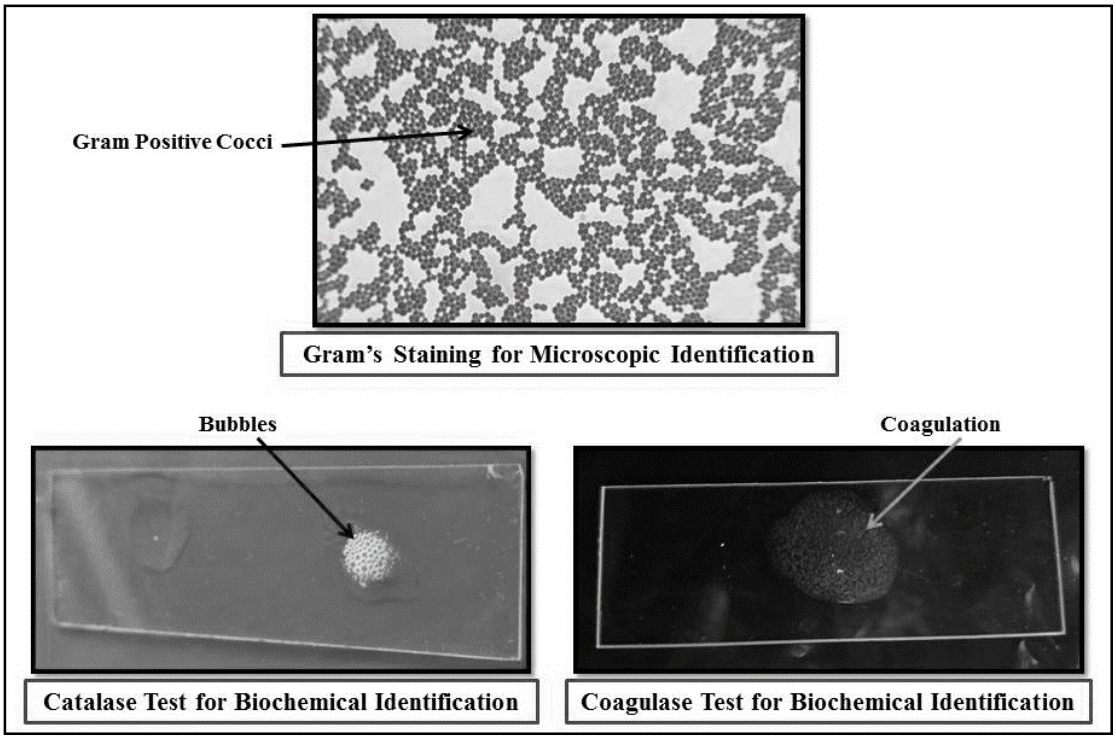

Fig. 3. Microscopic and biochemical identification for *S. aureus*.

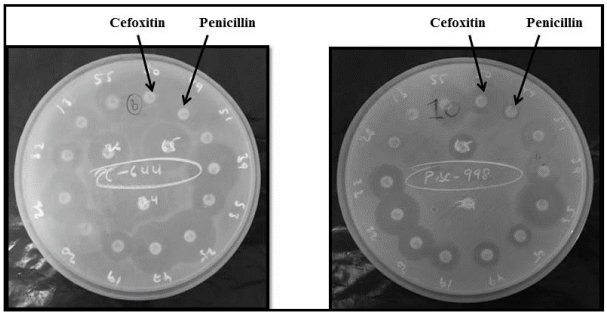

Fig. 4. Antibiotic susceptibility pattern by Kirby Bauer Method (Disk diffusion test) for MRSA identification (for MRSA - Penicillin G ≤ 28 mm and Cefoxitin ≤ 21 mm).

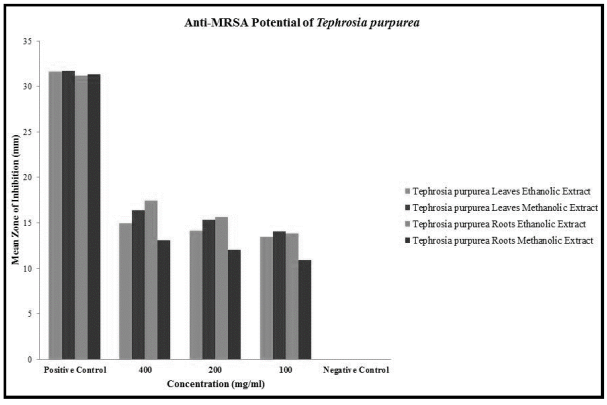

Fig. 5. Anti-MRSA activity of ethanolic and methanolic extracts of *Tephrosia purpurea* leaves and roots.

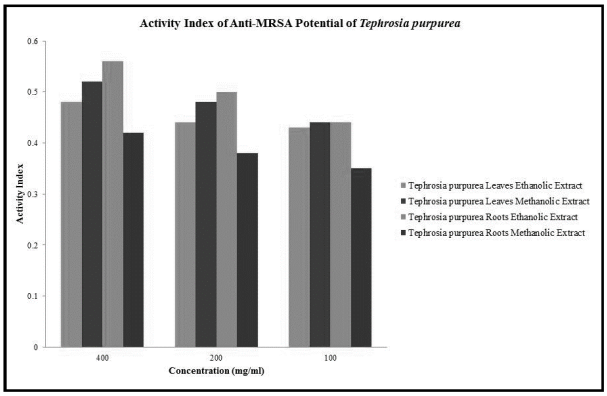

Fig. 6. Activity index of ethanolic and methanolic extracts of *Tephrosia purpurea* leaves and roots.

situation. One such plant is *T. purpurea*, which was screened for its anti-MRSA potentials and for the presence of phytochemicals, in the study. The results indicated that the ethanolic roots of *Tephrosia* exhibited the best antibacterial activity against the MRSA clinical isolates. As per the results of this study, it can be stated that the extracts of *T. purpurea* can be used as promising alternatives for the antibiotics. This study can be further planned to indicate the bioactive component that might be responsible for the plant's antibacterial activity and to identify the mechanisms which

Table 1. Anti-MRSA activity (mean zone of inhibition) of ethanolic and methanolic extracts of *Tephrosia purpurea* leaves and roots

		Positive control (in mm)	400 mg/ml (in mm)	200 mg/ml (in mm)	100 mg/ml (in mm)	Negative control
Tephrosia purpurea leaves	Ethanolic extract	31.61	15.000	14.15	13.484	0
	Methanolic extract	31.69	16.393	15.333	14.03	0
Tephrosia purpurea roots	Ethanolic extract	31.212	17.454	15.667	13.85	0
	Methanolic extract	31.333	13.121	12.000	10.94	0

Table 2. Activity index of ethanolic and methanolic extracts of *Tephrosia purpurea* leaves and roots

		400 mg/ml	200 mg/ml	100 mg/ml
Tephrosia purpurea leaves	Ethanolic extract	0.48	0.44	0.43
	Methanolic extract	0.52	0.48	0.44
Tephrosia purpurea roots	Ethanolic extract	0.56	0.50	0.44
	Methanolic extract	0.42	0.38	0.35

might be responsible for the antibacterial activity of the plant extracts.

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