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IN-VITRO ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACTS OF *HEMIDESMUS INDICUS* AND *SIMAROUBA GLAUCA*

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ABSTRACT: One of the biggest issues faced in today's health-care and medicine is the threat posed by the rapidly emerging drug-resistant microorganisms. Certain Enterobacteriaceae, *Pseudomonas aeruginosa, Staphylococcus aureus,* etc. are showing resistance against most of the known antibiotics. These organisms being pathogenic need to be contained, and prevented from causing infections. Since the 'Drugs of last Resort' are no longer effective, there is an urgent necessity to search for equally effective therapeutics. In the following study, ethanolic extracts of roots of *Hemidesmus indicus* and leaves of *Simarouba glauca* were evaluated for their antimicrobial activity against *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* by well-diffusion method. The minimum inhibitory concentration was determined. Further, the isolation and purification of the constituents responsible are under process.

KEYWORDS: Antimicrobials, Herbal treatment, Phytochemicals, Ethanol extracts, Drug-Resistance.

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1.INTRODUCTION

Sir Alexander Fleming, in the year 1928, discovered Penicillin-G or benzylpenicillin from a fungi *Penicillium notatum*, which was effective against Staphylococci spp., and many other infection causing bacteria. This penicillin drug belongs to a class known an antimicrobial or specifically antibiotics which are used in the prevention and treatment of bacterial infections. In the 20th century, antibiotics revolutionized medicine and healthcare and was thought to be one of the greatest inventions. However, this invention was not long lived, when bacteria starting evolving mechanisms to 'resist' the action of antibiotics. These organisms called 'Drug resistant bacteria' are more virulent

and poses greater challenges in the medical community. [1] [2]. Antibacterial agents vary in targets and are classified accordingly. These include inhibition of protein synthesis, inhibition of nucleotide synthesis and blocking certain important metabolic pathways. [3] The antibiotic penicillin was commercially produced by early 1940s and the first case of resistance to this drug was reported in 1965. Since then different classes of antibiotics have been discovered but due to rapid evolution – horizontal gene transfer and mutations, bacteria are becoming 'multi-drug' resistant. It is tough to manage such infections especially in immune-compromised patients. Apart from the organism's natural ability to resist the drug, overuse, inappropriate prescription usage in agriculture and fisheries are the main causes for this scenario.

Mechanisms of resistance include -

Changes in outer membrane permeability: some small drugs like quinolones diffuse through the porin channels into the bacteria. To acquire resistance some bacteria like *Pseudomonas aeruginosa* reduce the porins such that the drugs cannot diffuse. Efflux pumps: certain membrane proteins have the ability to expel low-concentration antibiotics as and when they enter the cells. These proteins are called efflux pumps. Macrolides and tetracycline are exported this way conferring resistance to the bacteria. Modification of target molecule: alteration and mutation in target proteins prevent it from being recognized by the antibiotics. Modified Penicillin Binding Proteins (PBP) gives resistance to *S. aureus*. Mutated DNA gyrase and Topoisomerase provide resistance against Quinolones. Antibiotic inactivation: more advanced strategies of antibiotic resistance includes enzymatic inactivation or degradation. Enzymes like Aminoglycoside-modifying enzymes, β-lactamases and Chloramphenicol-acetyl transferases hydrolyze aminoglycosides, β-lactam antibiotics and chloramphenicol respectively. [2][3][4] Some classical examples resistant bacteria include Vancomycin-resistant Enterococci (VRE), Multidrug-resistant *Pseudomonas aeruginosa*, Drug-resistant non-typhoid Salmonella, Drug-resistant Shigella, Methicillin-resistant *Staphylococcus aureus* (MRSA). [2] [4] Drug-resistance along with the ability of bacteria to exist in complex communities called 'biofilm' is making treatment, especially for critically-ill patients in intensive care units very challenging [16]. Thus, there is a need for alternate therapeutics to tackle this problem. At this rate of bacterial evolution, newly isolated or synthesized antimicrobial agents might not stay effective for long periods. Hence herbal-based treatments are gaining a lot of importance. [19] [22] Natural products have been used as remedies for various aliments since several centuries [25]. They contain secondary metabolites called phytochemicals present in different parts of the plant – roots, stem, leaves, etc. which have therapeutic properties. Some of these constituents include Alkaloids, Phenols, and Flavonoids, which are known to have anti-bacterial activity [27]. There chemicals, being naturally occurring tend to have fewer side-effects, much lesser toxicity and reportedly similar therapeutic effect as synthetic antibiotics. *Hemidesmus indicus* commonly called 'Indian Sarsaparilla', 'Nannari' and 'Anantmool' is a small shrub found in South Asia and

extensively in Southern India and Gangetic plains [23]. It belongs to Order Gentianales, Family Ascelepiadaceae, Genus *Hemidesmus* and Species *indicus*. *Hemidesmus indicus* is a short, slender lactiferous shrub. The roots and stem are twined in anticlockwise direction. It is woody and have characteristic, vanillin-like aroma [5]. Leaves are simple, dark green and petiolate. Flowers are small and range from greenish-yellow to purple. This plant possesses Phyto-constituents like glycosides, flavonoids, tannins and sterols [6]. The roots specifically have hemidesmol, resin, glucoside, Coumarins, Terpenoids and Saponin [7]. These shrubs have diverse ethnobotanical properties, some of which include: Antilithic [8], Antivenomous (for Scorpion stings and snake bites) [5], antiulcer, antidiabetic [6], anti-arthritic, antimicrobial [30], anticancer, anti-inflammatory, antileprotic and antioxidant activity [9]. Studies have also reported larvicidal potential of *Hemidesmus indicus* root extracts [10]. *Simarouba glauca* also called Bitter wood, Paradise tree and Lakshmi taru [24], belongs to Order Sapindales, Family Simaroubaceae, Genus *Simarouba* and Species *glauca*. It has tropic distribution and is found in Africa, America, Madagascar, Cuba, and Brazil and was introduced to India in the later part of 20th century [11]. *Simarouba glauca* is a medium sized tree and can grow in shade in the canopy of larger trees. The bark is dark and cracked on the outer surface. The leaves are compound, with 3-21 leaflets. These are pinnate, oblong in shape, smooth apex and entire margins. The leaflets are slightly waxy and dark green on the upper surface [11] [12]. The major chemicals present include alkaloids, flavonoids, glycosides, phenolic compounds, saponin, fixed oils and cardinolides [12]. *Simarouba glauca* has certain biological activities like Anti-amoebic, antibacterial [29], anticancer, anti-malarial, antioxidant, anti-fungal and anti-ulcer [12] [13]. These leaves also have hemolytic and anti-thrombotic activity [14]. The phytochemicals were isolated by cold maceration technique. The powdered plant sample was immersed with a suitable solvent in a stoppered container and allowed to stand for a fixed period of time, at room temperature with occasional stirring, but no violent agitation. [15] [19]. This study, therefore deals with the isolation and evaluation of the antibacterial activity of the extracts against *Staphylococcus aureus* [31]*, Bacillus subtilis* and *Escherichia coli.* [21] [26]

2. MATERIALS AND METHODS

Collection of Sample

Roots of *Hemidesmus indicus* and leaves of *Simarouba glauca* were purchased from a local vendor in Bangalore. The samples were authenticated to be root of *Hemidesmus indicus* and leaves *Simarouba glauca* by Dr. Mamatha A, Associate Professor, KLE College of Pharmacy, Bengaluru and the voucher specimens were deposited.

Pre-extraction procedure

The collected samples were then shade dried for about 10 days, tossing every 6 hours. The samples were then pulverized in an electric blender to coarse powder, which was then stored in air tight containers until used.

Extraction

Maceration - 10g of the plant powder was soaked in 100ml of distilled water and ethanol separately for 48 hours at room temperature with occasional stirring. Then the solution was filtered and the filtrate was concentrated by evaporation on water bath, allowed to dry and then stored in airtight container at 4°C.

Microbial study

Pure cultures of *Escherichia coli* (MTCC 739), *Staphylococcus aureus* (MTCC 737) and *Bacillus subtilis* (MTCC 441) were collected from KLE College of Pharmacy, Bengaluru and were cultured in Nutrient broth at 37°C for 24 hours. 150µL of this broth was spread on nutrient agar plates and colony count was taken.

Anti-bacterial activity

Briefly, 150µL of pure cultures of *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis* having 1×10^6 CFU/ml was spread on nutrient agar and bores were made using sterile borer. 50µL of sample of varying concentrations 5%, 2.5%, 1.25%, 0.625% and 0.3125%, standard 0.2% chlorhexidine and ethanol as controls were added. It was incubated at 37°C for 20 hours and zones of clearance was observed. Further the minimum inhibitory concentration was obtained. The trials were done in triplicates and the mean value and standard deviation was calculated.

3. RESULTS AND DISCUSSION

Microbial study

Table 1: Results for Microbial study of *S. aureus, B. subtilis* and *E. coli*

ORGANISM	CFU (Colony Forming Units)/ml
Staphylococcus aureus	2×10^6
Bacillus subtilis	7.2×10^6
Escherichia coli	1.2×10^6

The Colony Forming Units of *S. aureus, B. subtilis* and *E. coli* per mL of broth was 2×10^6, 1.2×10^6, and 7.2×10^6 respectively.

Anti-bacterial activity

Table 2: Zone of Inhibition (mm) – treatment with ethanolic extract of *Hemidesmus indicus*

Extract	Organism	Sample Concentration	Zone of inhibition (mm)	Standard - 0.2% chlorhexidine (mm)
Ethanolic	*S. aureus*	5%	16.5±2.5	18.33±1.25
		2.5%	-	
		1.25%	-	
	E. coli	5%	12±5.5	14.67±2.05
		2.5%	13	

		1.25%	24	
	B. subtilis	5%	12	17.67±3.30
		2.5%	14	
		1.25%	17	

For *Hemidesmus indicus* Ethanolic extracts were used at 1.25%, 2.5% and 5% concentration. There was no zone of inhibition against *S. aureus* at 1.25% and 2.5%. Whereas at 5% concentration, 16.5mm Zone of inhibition was seen. 12mm, 13mm and 24mm Zones of inhibition was seen at 5%, 2.5% and 1.25% respectively against *E. coli.* For *B. subtilis,* zones of 12mm, 14mm and 17mm was seen at 5%, 2.5% and 1.25% respectively. The experiment was conducted in triplicates. At 5% concentration of Ethanolic extract of *Hemidesmus indicus* all three organisms showed zones of inhibition. For *Simarouba glauca* Ethanolic extracts were used at 0.3125%, 0.625% 1.25%, and 2.5% and 5% concentration. In *S. aureus* plates, zone of inhibition of 22.5mm, 21.8mm, 20mm, 14mm and 15mm was seen for 5%, 2.5%, 1.25%, 0.625% and 0.3125% concentrations respectively. In *E. coli* plates, Zone of inhibition of 19.5mm, 14.8mm and 16.5mm was seen for 5%, 2.5% and 1.25% concentrations respectively. In *B. subtilis* plates, zone of inhibition of 20mm, 15.5mm, 15.5mm and 15mm was seen for 5%, 2.5%, 1.25% and 0.625% concentrations respectively.

Table 3: Zone of Inhibition (mm) – treatment with ethanolic extract of *Simarouba glauca*

Extract	Organism	Sample Concentration	Zone of Inhibition (mm)	Standard- 0.2% Chlorhexidine
Ethanolic	*S. aureus*	5%	22.5±2.59	19±1.41
		2.5%	21.83±2.26	
		1.25%	20±5	
		0.625%	14	
		0.3125%	15	
	E. coli	5%	19.5±2.95	17.8±4.11
		2.5%	14.8±2.49	
		1.25%	16.5±0.5	
		0.625%	-	
		0.3125%	-	
	B. subtilis	5%	20±2.54	17.2±2.56
		2.5%	15.5±2.62	
		1.25%	15.5±0.5	
		0.625%	15	
		0.3125%	-	

The experiment was conducted in triplicates. From the above results obtained, 5% ethanolic extract of *Hemidesmus indicus* and 1.25% ethanolic extract of *Simarouba glauca* were found to show significant in-vitro antibacterial activity against all three organisms chosen for the study. Preliminary qualitative phytochemical analysis shows the presence of Flavonoids, Alkaloids, Phenolic compounds and Coumarins which are all associated with anti-bacterial activity. Moreover, Alkaloids like Tomatidine and Cinchona Alkaloids; Sulphur-containing phytochemicals like Isothiocyanates and Allicin and Terpenoids like Carvacrol and Limonene are known to be effective against *Staphylococcus aureus* [31]. Similarly, against *Escherichia coli*, Alkaloids – Reserpine and Piperine; Allyl Isocyanates and Terpenoids like Thymol and citral are known to have potent bactericidal activity. [17] [18] [28]

Fig. 1: Zone of inhibition on *S. aureus* plate

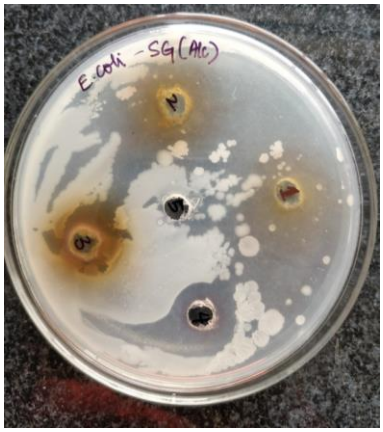

Fig. 2: Zone of inhibition on *E. coli* plate

Fig. 3: Zone of inhibition on *B. subtilis* plate.

4. CONCLUSION

The concern caused by the increasing rate of antibiotic resistant strains of microorganisms requires immediate alternative solutions. Plant extracts have been used for a very long time and have great potential as anti-bacterial agents. In this study, Ethanolic extracts of root of *Hemidesmus indicus* and leaves of *Simarouba glauca* were prepared and their antibacterial activity against *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* were evaluated. It can be concluded that, both plants possess significant antibacterial activity. Further, isolation and characterization of individual constituents of the plants responsible for the activity is being assessed.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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