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INVESTIGATION OF ANTIBACTERIAL ACTIVITY OF *SALACIA RETICULATA* (ROOT) AGAINST SOME IMPORTANT PATHOGENIC BACTERIA

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ABSTRACT:

Different solvent extract viz., petroleum ether , chloroform , methanol and ethanol extracts of root of *S. reticulate* was evaluated for antifungal activity against four different bacterial species viz., *Staphylococcus aureus* , *Escherichia coli, Bacillus subtilis* and *Pseudomonas* sp. among the four solvent extract tested, petroleum ether extract recorded a maximum inhibition against all the test bacteria tested at 500, 1000, 1500 and 2000 ppm concentration and compared to synthetic antibiotic chloramphenicol tested at 25mg concentration. Petroleum ether extract was subjected for TLC and the three fractions (each fraction consists of three bands) was identified and among three fractions, fraction II (with R_f value of the band 0.30, 0.33 and 0.36) recorded a maximum inhibition against all the test bacterium.

KEYWORDS: *S. reticulate*, antibacterial activity, fractions, bioactive principles.

INTRODUCTION: Infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Death from infectious diseases ranked 5th in 1981, has become the 3rd leading cause of death in 1992, with an increase 58% (Venkataswamy et al., 2010). To overcome this ill effects, the common procedure is to use synthetic antibiotics to manage these infectious pathogens. There has been an alarming increase in the incidence of new and re-merging infectious diseases, appearance of undesirable side effects of certain antibiotics, as well as the increasing development of resistance to the antibiotics in current clinical use (cowan, 1999). Bacterial resistance to antibiotics has been a recognized reality almost since the dawn of the antibiotic era, but only within the past twenty years has the emergence of dangerous, resistant strains occurred with a disturbing regularity. This escalating evolution of resistance coupled with a diminished antibiotic pipeline has led some to claim that a post-antibiotic era is eminent (Appelbaum , 2012; Richard , 2014). To avoid the usage of synthetic antibiotics, For several years now, plants and plant materials have been used as a source of medicinal agent and numerous natural products acquired from medicinal plants either as a crude extract or as purified products have been employed in disease control. Medicinal plant parts have been extensively used to extract raw drugs owing to possession of various medicinal properties. They constitute credible sources for a huge number of modern drugs, several of which are usually based on their traditional folk medicine (Khaleel, 2016). Herbal medicine use employed either in traditional medicine practice or complementary and alternative medicine (CAM) is popular for 80% percent of the world in Asia, Latin America, and Africa and is reported to have minimal side effects (Doughari, 2007). The search for alternative antimicrobial compounds is an urgent area of biomedical research and extracts derived from plants have long held interest as potential sources of new therapeutic agents (Cowan, 1999). Plants have been used as medicines from ancient times. Medicinal plants are rich sources of number of chemical ingredients which can be used in drug development and synthesis. Plants are important sources of nutrition and have therapeutic values (Hassan , 2012). In the present study, *Salacia reticulata* (root) commonly called salacia, kotalahimbata and marking nut tree belongs family hippocrateacaea were tested against five different bacterial species.

MATERIALS AND METHODS

Plant Material: Fresh and healthy roots of *S. reticulata* collected from Mysore. The roots were thoroughly washed two to three times with running tap water and one to two times with sterile distilled water. The washed

roots were air dried at room temperature on a sterile blotter, and used for the preparation of solvent extracts.

Test organisms: Five species of bacteria viz., *Staphylococcus aureus* (Gram positive), *Escherichia coli* (Gram Negative), *Bacillus subtilis* (Gram positive), and *Pseudomonas* sp. (Gram Negative) were collected from research center, CMR Institute of Management studies (Autonomous), Bangalore. The obtained cultures were sub cultured on nutrient agar medium. After 24 hours of incubation at 37°C, the cultures were preserved aseptically in refrigerator until further use.

Preparation of Solvent extraction: Thoroughly washed roots of *S. reticulata were* dried in shade for five days and then powdered with the help of Waring blender. 25 grams of shade dried powder was filled in the thimble and extracted successively with petroleum ether, chloroform, methanol and ethanol in a Soxhlet extractor for 48 hours. Solvent extracts were concentrated under reduced pressure. After complete evaporation, 1 gram of each concentrated solvent extracts were dissolved in 9 ml of methanol and used for antibacterial assay (Lalitha et al., 2011).

ANTIBACTERIAL ACTIVITY

Preparation of standard culture inoculums of test organism: Fresh culture of all the test bacterial species were inoculated into 2 ml of Nutrient broth and incubated at 37^0 C for 24 hours till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by WHO.

Solvent Extract: One gram of different solvent extract of *S. reticulata* roots were dissolved in 9 ml of methanol. The sterile nutrient agar medium in petridishes was uniformly smeared with test culture. 5 mm wells were made in each petridish to which 50µl of 500, 1000, 1500 and 2000ppm of different solvent extracts dissolved in methanol were added. For each treatment ten replicates were maintained. Respective solvents served as control. Standard chloramphenicol (25mg) was used to compare the efficacy of solvent extract against test organisms\ (Lalitha et al., 2011).

Separation of different fractions by Thin Layer Chromatography (TLC)

Preparation of TLC plates and separation of fractions: TLC Plates are thoroughly washed with detergent and water, rinsed with distilled water and allowed for draining. 25 grams of silica gel adsorbent was mixed with 60 to 70 ml of distilled water. The gap of the TLC applicator was adjusted to 0.25 mm using feeler gauge provided. Silica gel slurry was poured into spreader and with a single constant motion; the slurry was drawn along the plates. After spreading, the plates were incubated at 110°C to 120°C overnight and cooled in desiccators before use. On thin layer plates, gently mark the intended positions of samples with a clean pointed glass rod at one horizontal edge of the plate. The obtained concentrated solvents of Petroleum ether, Chloroform, Methanol and Ethanol were dissolved in 10 micro liters of their respective solvents and used for loading it into prepared TLC plates. All the loaded samples were eluted with methanol: chloroform extract in the ratio 7:3. After the elutent were run for more then $3/4^{th}$ of the TLC plates, the plates were removed and examined under normal, short wavelength UV (254 nm) and long wavelength UV (366 nm) light in UV chamber. The obtained bands were divided into three fractions and each band consists of three bands. R_f value of each band were calculated using the formulae Distance moved by compound / Distance moved by solvent system and used further for antifungal activity (Sadasivam and Manickam, 2000).

Separation of different fractions: After obtaining different fractions, the R_f value was identified and carefully each bands were scraped and dissolved in chloroform and passed through Whatman No.1 filter paper and the collected filtrate was subjected for evaporation. After complete evaporation the obtained bioactive compound was collected and yield was calculated. The same procedure was followed for all the solvents.

Solvent Extract: One gram of different solvent extract of *S. reticulata* roots were dissolved in 9 ml of methanol and for each treatment, ten replicates were maintained. Respective solvents served as control. Standard antibiotic chloramphenicol (25mg) was used to compare the efficacy of solvent extract against test organisms.

Antibacterial activity of different fractions: The bioactive compound after collection was dissolved in chloroform and different concentrations viz., 500ppm, 1000ppm 1500ppm and 2000ppm were made. The sterile nutrient agar medium in petridishes was uniformly smeared with test bacterial culture. 5 mm wells were made in each petridish to which 50µl of 500, 1000, 1500 and 2000ppm of different solvent extracts dissolved in methanol were added. Nutrient agar medium with the same concentrations of these respective solvents served as control. The plates were incubated at 35±1°C for 24 hours and zone of inhibition if any around the well were measured in millimeter (mm) (Bansal and Guptha, 2000).

RESULTS

Antibacterial activity of Solvent extract: Among the four solvent extract viz., petroleum ether chloroform , methanol and ethanol tested at 500, 1000, 1500 and 2000 ppm concentration, maximum inhibition was observed in petroleum ether extract and moderate activity was observed in methanol extract. In petroleum ether extract, *S. aureus* recorded 9.0mm inhibition at 500ppm, 15.0mm at 1000ppm, 23.0mm at 1500ppm and 30.0mm at 2000ppm concentration. In *E. coli*, the percent inhibition was 7.0, 14.0, 19.0 and 26.0mm at 500, 1000, 1500 and 2000ppm. *B. subtilis* recorded 9.0, 16.0, 24.0 and 29.0mm inhibition. In *Pseudomonas* sp maximum

inhibition of 32.0mm was recorded at 2000ppm, in 500, 1000 and 1500ppm concentration, the inhibition was 18.0, 26.0 and 32.0mm respectively. Compared o synthetic antibiotic chloramphenicol, tested at 25mg concentration, *S. aureus* recorded 30.0mm, *E. coli* recorded 32.0mm, *B. subtilis* recorded 32.0mm and *Pseudomonas* sp recorded 32.0mm inhibition respectively.

In methanol extract, moderate activity was observed in all the concentration tested and recorded 15.0 mm inhibition in *S. aureus* at 2000ppm and least inhibition was observed in *B. subtilis* and recorded 2.0mm inhibition at 500ppm concentration. In 1000 and 1500ppm concentration, the average inhibition was 5.0mm to 11.0mm. No activity was observed in chloroform and ethanol extract.(Table 1 and 2).

Separation of different fractions by Thin Layer Chromatography: Based on the result of antibacterial activity, maximum and significant activity was observed in petroleum ether extract. Hence the isolation of bioactive compound and separation of fractions was conducted in petroleum ether extract. Among the three fractions isolated, fraction I showed three bands and recorded the R_f value 0.22, 0.24 and 0.27. Fraction II recorded the R_f value of 0.30, 0.33 and 0.36. Fraction III recorded the R_f value of 0.39, 0.42 and 0.48 respectively (Figure 1).

Antibacterial activity of different fraction: Among the three fractions tested at 500, 1000, 1500 and 2000ppm concentration, fraction II recorded an maximum inhibition in all the test bacteria. *S. aureus* and *Pseudomonas* sp recorded a maximum inhibition of 32.0mm ant 2000ppm concentration. At 500ppm 1000ppm and 1500ppm concentration, *S. aureus* recorded 17.0, 26.0 and 32.0mm inhibition and *Pseudomonas* sp recorded 18.0, 26.0 and 32.0mm inhibition respectively. *E. coli* recorded10.0, 16.0, 23.0 and 19.0mm inhibition at 500, 1000, 1500 and 2000ppm concentration. *B. subtilis* recorded 9.0, 16.0, 24.0 and 30.0mm inhibition at 500, 1000, 1500 and 2000ppm concentration (Table 3). No activity was observed in Fraction I and Fraction III.

Table 1: Antibacterial activity of petroleum ether and chloroform extract of root of *S. reticulate*

Bacteria	Solvent extract								Synthetic antibiotic Chloramphenicol (25mg)
	Petroleum ether				Chloroform				
	Concentration of the plant extract								
	500 ppm	1000 ppm	1500 ppm	2000 ppm	500 ppm	1000 ppm	1500 ppm	2000 ppm	
Staphylococcus aureus	9.0^{a} ±0.0	15.0^{b} ±0.0	23.0^{c} ±0.1	30.0^{d} ±0.1	-	-	-	-	30.0^{a} ±0.0
Escherichia coli	7.0^{a} ±0.1	14.0^{b} ±0.0	19.0^{c} ±0.0	26.0^{d} ±0.1	-	-	-	-	32.0^{b} ±0.1
Bacillus subtilis	9.0^{a} ±0.0	16.0^{b} ±0.0	24.0^{c} ±0.0	29.0^{d} ±0.0	-	-	-	-	32.0^{b} ±0.0
Pseudomonas sp.	10.0^{a} ±0.1	18.0^{b} ±0.0	26.0^{c} ±0.0	32.0^{d} ±0.0	-	-	-	-	32.0^{b} ±0.0

- Values are the mean of five replicates, ±standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

Table 2: Antibacterial activity of methanol and ethanol extract of *S. reticulate* root

Bacteria	Solvent extract								Synthetic antibiotic Chloramphenicol (25mg)
	Methanol				Ethanol				
	Concentration of the plant extract								
	500 ppm	1000 ppm	1500 ppm	2000 ppm	500 ppm	1000 ppm	1500 ppm	2000 ppm	
Staphylococcus aureus	4.0^{a} ±0.0	7.0^{b} ±0.0	11.0^{c} ±0.0	15.0^{d} ±0.1	-	-	-	-	30.0^{a} ±0.0
Escherichia coli	3.0^{a} ±0.0	6.0^{b} ±0.0	10.0^{c} ±0.1	13.0^{d} ±0.0	-	-	-	-	32.0^{b} ±0.1
Bacillus subtilis	2.0^{a} ±0.1	5.0^{b} ±0.1	7.0^{c} ±0.0	10.0^{d} ±0.1	-	-	-	-	32.0^{b} ±0.0
Pseudomonas sp	3.0^{a} ±0.0	5.0^{b} ±0.0	8.0^{c} ±0.0	11.0^{d} ±0.0	-	-	-	-	32.0^{b} ±0.0

- Values are the mean of five replicates, ±standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

Table 3: Separation of fractions and determination of R_f value of Petroleum ether extract of *S. reticulate* root

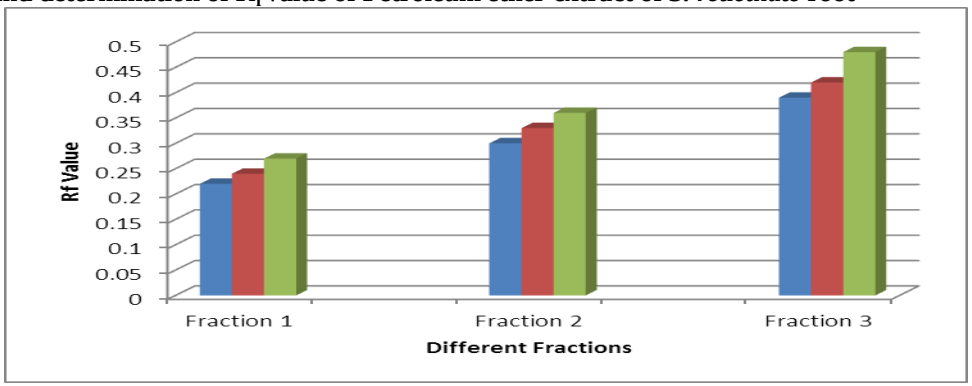

Table 4: Antibacterial activity of different fractions of petroleum ether extract of *S. reticulate* root

Fungi	Petroleum ether extract												Synthetic antibiotic Chloramphenicol (25mg)
	Fraction I				Fraction II				Fraction III				
	Concentration of the plant extract												
	500 ppm	1000 ppm	1500 ppm	2000 ppm	500 ppm	1000 ppm	1500 ppm	2000ppm	500ppm	1000ppm	1500ppm	2000ppm	
Staphylococcus aureus	-	-	-	-	11.0^{a} ±0.0	17.0^{b} ±0.0	26.0^{c} ±0.0	32.0^{d} ±0.0	-	-	-	-	30.0^{a} ±0.0
Escherichia coli	-	-	-	-	10.0^{a} ±0.0	16.0^{b} ±0.1	23.0^{c} ±0.0	29.0^{d} ±0.0	-	-	-	-	32.0^{b} ±0.1
Bacillus subtilis	-	-	-	-	9.0^{a} ±0.0	16.0^{b} ±0.0	24.0^{c} ±0.0	30.0^{d} ±0.1	-	-	-	-	32.0^{b} ±0.0
Pseudomonas sp	-	-	-	-	10.0^{a} ±0.1	18.0^{b} ±0.1	26.0^{c} ±0.1	32.0^{d} ±0.0	-	-	-	-	32.0^{b} ±0.0

- Values are the mean of five replicates, ±standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

DISCUSSION: Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country (Bishnu etal.,2009). Problems associated with topical fortified antibiotics such as local toxicity, stability, cost, need for refrigeration and the emergence of antibiotic-resistant organisms have prompted interest in the search for therapeutic alternatives. The increasing prevalence of multi drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infections fighting strategies (Sieradski etal., 1999; Nagendra et al., 2010). From the above result, it was observed that, in solvent extract, maximum activity was observed in petroleum ether extract and loderate activity was observed in methanol extract. No activity was observed in chloroform and ethanol extract. Hence the bioactive compound was rich in petroleum ether extract and further isolation of bioactive compound was conducted and among three fractions (I, II and III), Fraction II recorded a maximum antibacterial activity. Antibacterial activity of solvent extract of *S. reticulata* root was not much evaluated based on the survey of literature.

CONCLUSION: From the above observation and result, it was concluded that, Fraction II recorded a highly significant activity in inhibiting all the test bacterial species. Further research is necessary to identify the bioactive principle responsible for antibacterial activity and its characterization by subjecting the bioactive compound to standard protocol. The bioactive compound will be a promising source for developing a drug formulation which is eco-friendly.

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