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Invitro Study of Antibacterial Activity of Leaf and Root Extract of Rauvolfia Serpentina against Gram Positive and Negative Bacterial Strains

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Abstract: **In this study Methanolic and chloroform leaf and root extract of Rauvolfia serpentina was studied for its antibacterial activity. Antibacterial activity of leaf and root extracts was assessed against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus* subtilis and *Klebsiella pneumonia* by disc diffusion method. Methanolic extract of root was showed the maximum zone of inhibition for all test organisms than the leaf extract. According to observations of root extract of 50µl/ml concentration 15.4mm, 16.2mm, 12.3mm,10.1mm and 15.0mm zones of inhibition and for concentration of 100µl/ml 22.5mm, 23.1mm, 15.1mm, 18.0mm, 22.0mm zones of inhibition were formed against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Klebsiella pneumonia* respectively. 50µl/ml concentration of leaf and root chloroform extracts showed no zone of inhibition against *Staphylococcus aureus* and *Bacillus subtilis,* maximum zone of inhibition was observed 15.0mm and 15.5mm against *E. coli* for leaf and root chloroform extract respectively. 100µl/ml concentration showed maximum zone of inhibition against all test organisms for both leaf and root extracts. All the bacteria were more susceptible to methanolic extracts than the chloroform extracts.**

Keywords: **Methanolic, antibacterial activity, Disc diffusion method, Zone of inhibition, Rauvolfia serpentine.**

1. INTRODUCTION

Plants are oldest source for bioactive substances used as pharmaceuticals, food additives, colors, flavors etc. In India, many indigenous plants are used as medicines to cure diseases and heal wounds and injuries. Plants gaining more importance as medicines because of presence of chemical substances such as alkaloids, carbon compounds, hydrogen, nitrogen, glycosides, essential oils, fatty oils, resins, mucilage, tannins, gums and others. These potent bioactive compounds are used for therapeutic purpose. Medicinal plants are gaining more importance because the shift of view and attitudes of people's mind towards the natural drugs. *Rauvolfia serpentina* is flowering plant belongs to family Apocynaceae and is native to the Indian Subcontinent and East Asia. *Rauvolfia serpentina* is an important medicinal plant of the world and widely used in both modern medical system and Ayurveda medical system. It is one of the 50 fundamental herbs used in traditional Chinese medicine. The plant is commonly known as Sarpagandha, Chandrabagha, Snake root plant, Chotachand, Chandrika and Harkaya etc [1] More than 50 alkaloids have been reported from Rauvolfia serpentina. These alkaloids are classified into 3 groups, reserpine, ajmaline and serpentine groups. Reserpine group comprises of reserpine, rescinnamine, deserpine etc. Ajmaline, ajmalicine, ajmalinine, iso-ajmaline etc. are of the ajmaline group, whereas, serpentine group includes serpentine, sepentinine, alstonine etc. The roots, leaves and juice of *R.*

serpentina contain a large number of secondary metabolites and has been used in Ayurvedic medical system for the treatment of various ailments [2]-[4]. In Ayurveda the roots of *R. serpentina* is used for curing hypertension, insomnia, mental agitation, gastrointestinal disorders, excitement, epilepsy, traumas, anxiety, excitement, schizophrenia, sedative insomnia and insanity [2], [4]. Plant extracts is used to cure circulatory disorders [5]. The root juices or extract is used to treat liver and abdomen pain, various gastrointestinal disorders and to expel intestinal worms from the children [6] –[8]. Plant is also used to treat snake bite by certain local people [9]. The other diseases such as pneumonia, malaria, body aches, eczema, burns, menstrual disorders, scabies, skin cancers, asthma, respiratory problems, eye inflammation, spleen diseases and fever can also be cured using *R. serpentina* [10]- [17]. Extracts of plants also show antioxidant [18], [22], Antidiarrhoeal activity [19], antitumor activity [20], antimicrobial and antiproliferative activities [21], [22] and Hypoglycemic and hypolipidemic activities [23]. This plant is also used for the treatment of high blood pressure [24]. Plant extracts possess antibacterial and antifungal activity [25]- [30]

2. MATERIAL AND METHODS

Antibacterial activity of root and leaf extracts of Rauvolfia serpentina was assessed against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Klebsiella pneumoniae

Extraction:

The collected leaves and roots were washed with distilled water and separated from undesirable materials. The washed leaves and roots were dried at room temperature for seven days under shade. After drying the materials were powdered separately. 50 grams of each dried powder was extracted with 100 ml of methanol [80%] and chloroform successively up to 48h. The mixtures were filtered with Whatman filter paper. The filtrates obtained were evaporated by rotary flash evaporator at 5 to 6 rpm and 65^{o}C temperature. After complete solvent evaporation, one gram of each concentrated solvent extracts were dissolved in 9ml of distilled water and stored at refrigerator (4^{o} C) for further use.

Test Microorganisms: Pure cultures of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Klebsiella pneumoniae were obtained from Department Microbiology and Biotechnology, Jnanabharathi Campus, Bangalore University, Bangalore

Inoculum preparation: 10 ml of distilled water was taken in test tube and one loopfull pure colony of freshly cultured bacteria of the experimental species were added into test tubes. OD was measured to confirm the population density of 100-110 per ml.

Test solution preparation: One gram of plant leaf and root methanol and chloroform extracts were dissolved in 9ml of distilled water. Crude extracted discs were prepared in different concentrations [50µl/ml and 100µl/ml] for both methanol and chloroform extracts.

Disc diffusion method: The sterile nutrient agar medium was poured into petridishes and uniformly spread. Then 0.1 ml of test cultures was uniformly spread over agar medium. Sterile prepared discs were placed on agar plates and incubated at 37^{o}c for 36 hours. The plates were observed for the zone of inhibition. Zone of inhibition was measured using antibiotic zone scale.

3. RESULTS AND DISCUSSION

Antibacterial activity of methanol extract of leaf and root was estimated by measuring the zone of inhibition in mm for different bacterial species and zone of inhibition was recorded in table-1. Antibacterial activity for chloroform extract was recorded in table 2.

Table 1: Zone of inhibition in mm for 80% methanol extraction

Test organisms	Control	Leaf extract		Root extract	
		50µl/ml	100µl/ml	50µl/ml	100µl/ml
Staphylococcus aureus	0.0	14.6	20.7	15.4	22.5
Escherichia coli	0.0	15.3	21.4	16.2	23.1
Pseudomonas aeruginosa	0.0	10.0	13.3	12.3	15.1
Bacillus subtilis	0.0	4.0	10.0	10.1	18.0
Klebsiella pneumoniae	0.0	14.8	21.0	15.0	22.0

Table 2: Zone of inhibition in mm for chloroform extraction

Test organisms	Control	Leaf extract		Root extract	
		50µl/ml	100µl/ml	50µl/ml	100µl/ml
Staphylococcus aureus	0.0	0.0	10.5	0.0	11.0
Escherichia coli	0.0	15.0	21.1	15.5	22.0
Pseudomonas aeruginosa	0.0	10.0	11.7	11.0	13.0
Bacillus subtilis	0.0	0.0	7.4	0.0	3.9
Klebsiella pneumoniae	0.0	10.2	11.8	11.0	12.6

Methanolic extract of leaves and roots of Rauvolfia serpentina exhibited the best antibacterial activity against all test organisms than the chloroform extract. Methanolic extracts of root were showed the maximum zone of inhibition for all test organisms than the leaf extract. According to observations of root extract of 50µl/ml concentration 15.4mm, 16.2mm, 12.3mm,10.1mm and 15.0mm zones of inhibition and for concentration of 100µl/ml 22.5mm, 23.1mm, 15.1mm, 18.0mm, 22.0mm zones of inhibitions were formed against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis,* and *Klebsiella pneumonia* respectively. Maximum zones of inhibition were recorded for 100µl/ml concentration for all test organisms. The degree of zone of inhibition varies from lowest 15.1mm to maximum 23.1mm against *Pseudomonas aeruginosa* and *Escherichia coli* respectively. 50µl/ml was not sufficient enough to inhibit *Pseudomonas aeruginosa*, and *Bacillus subtilis*, but suppress the growth [31]. Root bark acts as antidiarrhoeic agent by triple pronounced antibacterial, antiamoebic and antispasmodic action [32]. The antibacterial activity of root extracts was not because of any one main active principle but due to the combined action of additional other compounds [33]. Leaf extract of 50µl/ml concentration 14.6mm, 15.3mm, 10.0mm, 4.0mm, 14.8mm, zones of inhibition and for concentration of 100µl/ml 20.7mm, 21.4mm, 13.3mm, 10.0mm, 21.0mm zones of inhibitions were formed against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Klebsiella pneumonia* respectively. 50µl/ml concentration showed lesser zone of inhibition and minimum 4mm for *Bacillus subtilis* and maximum 15.3mm for *Escherichia coli*. 100µl/ml concentration showed minimum 10.0mm for *Bacillus subtilis* and maximum 21.4mm for *Escherichia coli.*

Chloroform extract of leaf and root showed varied degree of antibacterial activity against test organisms. 50µl/ml concentration of leaf and root extracts showed no zone of inhibition against *Staphylococcus aureus* and *Bacillus subtilis*, maximum zone of inhibition was observed 15mm and 15.5mm against E. coli for leaf and root extract respectively. 100µl/ml concentration showed zone of inhibition against all test organisms for both leaf and root extracts. Minimum zone of inhibition was recorded for Bacillus subtilis for leaf and root extracts and maximum was observed for E. coli 21.1mm and 22.0mm respectively. Deshwal and Vig reported antimicrobial activity of ethanol extract of R. serpentina [34]. Ahmed et al also reported antibacterial activity of extract of leaf, root of Rauvolfia serpentina against Escherichia coli, Salmonella typhi, and Pseudomonas aeruginosa [35]. Study of Jigna et al. reported that aqueous extract of R. serpentina L. recorded less antimicrobial activity as compared to ethanolic activity [36]. Hina fazal et al showed that ethanolic extract of roots of Rauvolfia serpentina exhibited the best antibacterial activity against B. subtilis [37] and according to Jinga et al against *B. cereus, B. subtilis, K. pneumonia* and *P. aeruginosa* [36].

All the bacteria were more susceptible to methanolic extracts than the chloroform extracts. Extracts were more effective against bacteria than fungi. All extracts tested for antibacterial potential showed varying degree of antibacterial activities against the Gram-positive, Gram-negative bacteria species. The best activity was recorded for the 100µl/ml concentration of methanolic extract of root of *Rauvolfia serpentina* against Gram negative bacteria E. coli which formed 23.1 mm zone of inhibition, followed by the chloroform extract 22.0mm zone of inhibition. This could be attributed to the higher concentration of the active substance causing the inhibitory effect was more in the root.

4. CONCLUSION

From this study it is concluded that among the extract of leaf and root of *R. serpentina*, root extract possess greater antibacterial activity against tested bacteria than extract of leaf. Our results demonstrated that methanol extracts possess greater antimicrobial activity than the other organic extracts [chloroform]. The tested extracts showed the better activity against Gram positive and Gram negative bacteria. The maximum antibacterial activity was measured for Gram negative

bacteria than Gram positive bacteria. However, further experimental studies are needed to elucidate the exact mechanism of action of antimicrobial activity to identify the active ingredients which can be used in drug development programs.

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