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Antimicrobial Activity of Chloroform and Methanolic Leaf Extract of Odina Wodier Roxb

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Abstract

The leaves of Odina wodier Roxb were collected from the forest in Karnataka district dried, powdered and extracted with Pet ether, chloroform, ethyl acetate and methanol. These crude extracts were tested for antimicrobial activity by the agar diffusion method. The extracts found to be active were subjected to, the extracts were prepared according to the MIC, and an antimicrobial susceptibility test was carried out using the agar well diffusion method. All the extracts showed antimicrobial activity against the tested strains. But the polar extracts showed a greater antibacterial potential as compared to the non-polar extracts. The methanolic extract was the most active. The present study showed the effectiveness of the crude plant extract against the tested bacterial strains. It indicated the potential use of the extract as an antimicrobial agent for the control of infectious diseases.

Keywords - *Odina wodier Roxb, Well diffusion assay, Antimicrobial activity.*

I. INTRODUCTION

In developing countries, particularly in India, low-income people such as farmers, people of small isolated villages, and native communities use folk medicine to treat common infections. These plants are ingested as decoctions, teas and juice preparations to treat the respiratory infection. They are also made into a poultice and applied directly to the infected wounds or burns. When people from these remote communities get an infectious disease, they are usually treated by traditional healers and shamans because of their expertise in such procedures as diagnosis, treating wounds, setting bones and making herbal medicines. Traditional healers claim that their medicine is cheaper and more effective than modem medicine. Patients of these communities have a reduced risk of getting infectious diseases from resistant pathogens than people from urban areas treated with traditional antibiotics. However, if they are treated in a hospital, the chance of contracting a nosocomial infection is increased. One way to prevent pathogenic species' antibiotic resistance is by using new compounds that are not based on existing synthetic antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient in treating infectious diseases than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant.

The plant Odina Wodier Roxb is a moderate-sized or large deciduous tree, with thick, soft branchlets belonging to Anacardiaceae. This tree occurs in hotter parts of India.

The decoction of the bark is used as astringent in cases of atonic dyspepsia and general debility. It is also used as a gargle in aphthous conditions of the mouth and toothache and as a lotion for skin eruptions. Fresh juice of the bark is used to sore eyes and obstinate ulcers. Powdered bark mixed with neem oil is an application for chronic ulcers and skin diseases and used as a paste for leprous ulcers. Gum of the tree made into an ointment with coconut milk or into liniment with brandy is a good application to sprains and bruises. Internally, the gum is given in asthma and as a cordial to women during lactation. Leaves boiled in oil are also applied to sprains and bruises to local swellings and the body's pains. For rheumatism, a paste of the leaves mixed with black pepper is a useful application.

The purpose of this study is to investigate the antimicrobial properties of Odina wodier Roxb. In this paper, we report the above study to investigate the finding of better and safe antimicrobial phytocompounds.

II. MATERIALS AND METHODS

A. Collection of plant material

The fresh plant leaves were collected in October from the Chamundeswari hills, Mysore, India. It was duly identified by the forest revenue officer Mrs.Vinutha DRFO (Deputy Range forest officer), and authentified by Botanist Dr. Gurukar Mathew, HOD Department of Botany, Bharathi College. After collection, the leaves were washed thoroughly with running tap water, cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanical

grinder. The voucher specimen was preserved in the laboratory for further reference.

B. Preparation of plant extracts

The collected plant parts were immediately brought to the laboratory, washed with tap water, surface sterilized with 10% sodium hypochlorite solution and rinsed with sterile distilled water and shade dried. After shade drying, the leaves were packed in brown cover and kept in an oven at 60˚C for an hour to make grinding easy. The samples were ground into powder using an electric blender. Two hundred grams of powder of leaves of Odina wodier Roxb were loaded in separate Soxhlet apparatus and extracted with solvents, petroleum ether, chloroform, ethyl acetate and methanol. The solvent was evaporated using a rotary evaporator under reduced pressure at 40˚ C, and the crude extracts were kept at 4˚C in a refrigerator for antimicrobial screening.

III. ANTIMICROBIAL ASSAY

A. Collection of test organisms

Staphylococcus aureus, Bacillus Subtalis, Escherichia coli, Pseudomonas aeruginosa, Candida Albicans, Mycobacterium bacillus were procured from JSS College of Pharmacy, Ooty, India and are used for determining antimicrobial activity.

B. Preparation of inoculum

Each organism was recovered for testing by sub-culturing on fresh media. A loopful inoculum of each bacterium was suspended in 5 ml of nutrient broth and incubated overnight at 37oC. These overnight cultures were used as inoculum. The growth of media employed in the present study included nutrient agar and nutrient broth. The medium was adjusted to pH 7.4 and sterilized by autoclaving at 15 lbs pressure (121oC) for 15 minutes.

C. Sub-culturing of microorganism

The pure cultures of microorganisms were maintained on nutrient agar slants by frequent subculturing. These cultures were stored at 4°C.

D. Preparation of Inoculums

Suspension of the organism was prepared as per McFarland standard. A 24 hr old culture was used for the preparation of the bacterial suspension. Suspension of the organism was made in a sterile isotonic solution of sodium chloride (0.9%w/v), and the turbidity was adjusted such that it contained approximately $1.5X10^8$cells/ml. It was obtained by adjusting the bacterial suspension's optical density equivalent to a mixture of 0.05ml of 1.175% of barium chloride and 9.95ml of 1% Sulphuric acid.

E. Procedure

Nutrient Agar medium was prepared using distilled water and subjected to sterilization in an autoclave at 121^0C for 1 hour. The Petri plates were washed thoroughly and sterilized in a hot air oven at 160^0C for 1 ½ hour. 30ml of sterile molten Nutrient agar medium was seeded with organisms (about 5ml according to McFarland's standard) in semi-hot conditions (40^0C). It was poured aseptically in a sterile Petri plate and allowed to solidify at room temperature. Bores were made on the medium using sterile borer, and 100-500 μl of the extracts at a different concentration were added to respective bore and Streptomycin at a concentration of 100µg/ml was taken as a standard and DMSO was used as a negative control. The Petri plates were incubated at 37 ± 2^0c for 24 hrs in a BOD incubator, and the zone of inhibition was observed and measured in mm using the scale. The results are given in **Table No 1-3.**

F. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations of the crude extracts were tested in Muller Hinton Broth for bacteria and Sabouraud Dextrose Broth for mycelia fungi to get the concentrations of 1000 –15.6 µg/ml by the broth macro dilution method. The culture tubes were incubated at 37 °C for 24 h (for bacteria) and 28 °C for 48 h (for fungi).

IV. RESULTS

In the present study, various concentrations of chloroform and methanol extracts of leaves of *Odina wodier* Roxb were tested against gram-positive bacteria *viz., Staphylococcus aureus,* and *Bacillus subtilis,* gram-negative bacteria *viz., Pseudomonas aeruginosa* and *Escherichia coli* and fungal strains such as *Candida albicans, Aspergius Niger* and *C. glabrata.* The Isolated test organisms exhibited the antimicrobial activity for different concentrations like 100 µg, 200 µg, 300 µg, 400 µg and 500 µg of different solvent extracts of *Odina wodier Roxb***.** The results are recorded in the following tables (1-3)**.**

Table 1: Choloroform extract

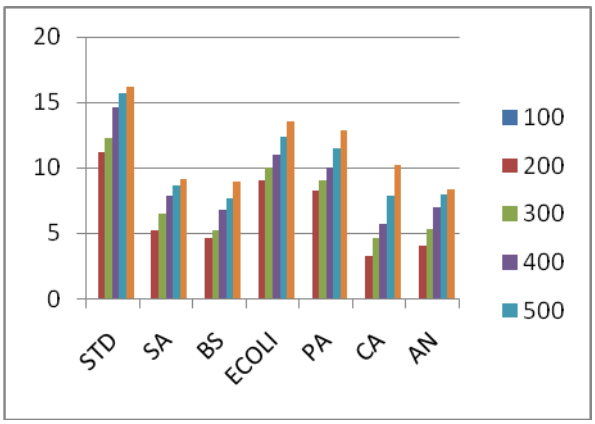
Sl no	Conc (µg/ml)	Streptomycin*	*Staphylococcus aureus**	*Bacillus Subtalis*	*Escherichia coli**	*Pseudomonas aeruginosa**	Candida albicans	Aspergillus niger.	MIC **(µg/mL)**
1	100	11.2	5.15	4.6	9	8.2	3.2	4	50
2	200	12.2	6.5	5.2	10.0	9	4.6	5.32	150
3	300	14.5	7.86	6.81	11	10	5.7	6.92	150
4	400	15.6	8.66	7.63	12.33	11.5	7.8	7.93	50
5	500	16.2	10.14	8.92	13.55	12.83	12.4	8.3	150

Table 2: Ethyl acetate extract

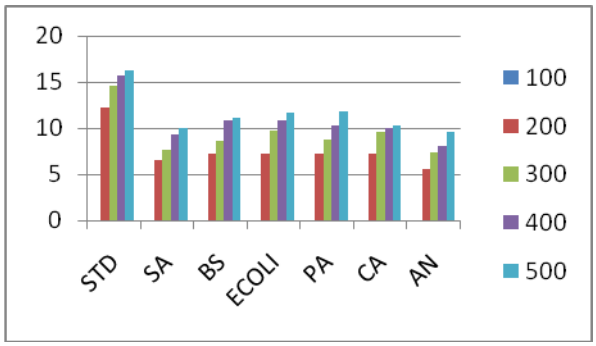
Sl no	Conc (µg)	Streptomycin*	*Staphylococcus aureus**	*Escherichia coli**	*Bacillus. Subtalis**	*Pseudomonas aeruginosa**	Candida albicans	Aspergillus niger.	MIC **(µg/mL)**
1	100	11.2	4.35	5.90	5.72	6.35	6.82	4.6	25
2	200	12.23	6.58	7.2	7.26	7.21	7.21	5.52	50
3	300	14.56	7.65	8.61	9.68	8.69	9.52	7.39	100
4	400	15.68	9.26	10.79	10.82	10.30	10.01	8.02	50
5	500	16.2	12.6	11.1	11.73	11.86	10.26	9.6	150

Table 3:Methanolic extract

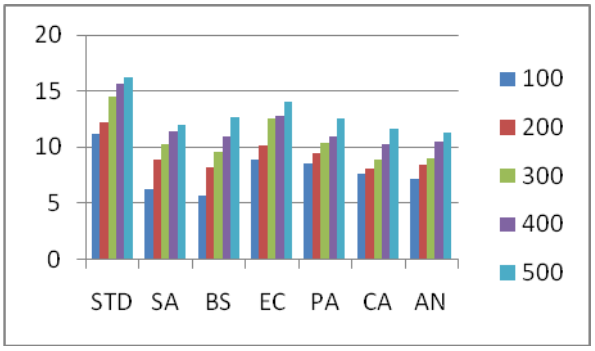
Sl no	Conc (µg)	Streptomycin*	*Staphylococcus aureus**	*Escherichia coli**	*Bacillus. Subtalis**	*Pseudomonas aeruginosa**	Candida albicans	Aspergillus niger.	MIC **(µg/mL)**
1	100	11.2	6.23	8.90	5.67	7.62	7.2	5.6	50
2	200	12.23	8.91	10.2	8.23	8.12	8.51	7.52	25
3	300	14.56	10.28	12.61	9.62	8.96	9.0	8.39	150
4	400	15.68	11.46	12.79	10.92	10.3	10.5	9.02	150
5	500	16.2	13.05	14.1	12.7	11.68	11.26	10.6	150



Graph 1: Chloroform extract



Graph 2: Ethyl acetate



Graph 3: Methanolic extract

Plates: Antimicrobial activity of methanolic extract of leaves of *Odina wodier* Roxb

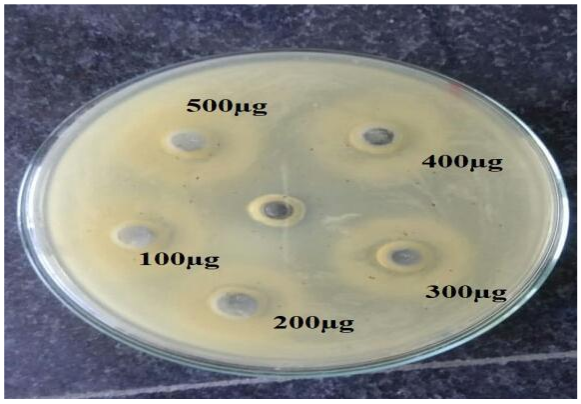


Fig 1: Zone of inhibition for Standard Streptomycin sulphate

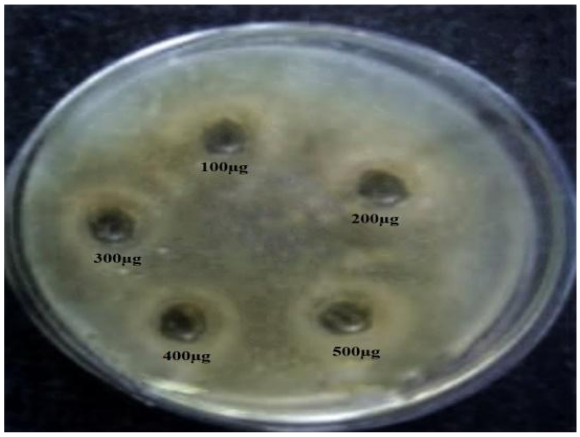


Fig 2: Zone of inhibition for Staphylococcus aureus

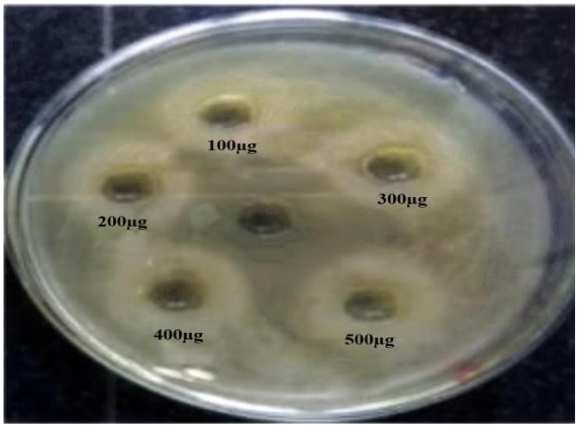


Fig 3: Zone of inhibition for Bacillus substalis

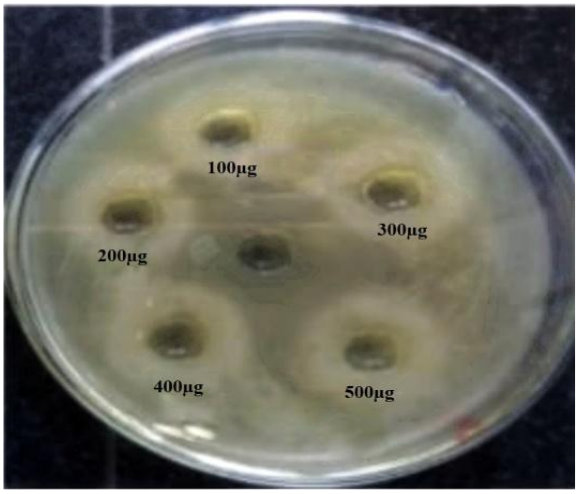


Fig 4: Zone of inhibition for Staphylococcus aureus

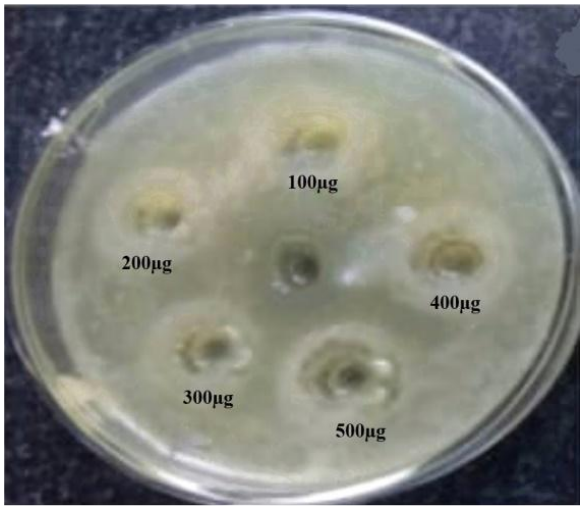


Fig 5: Zone of inhibition for Escherichia coli

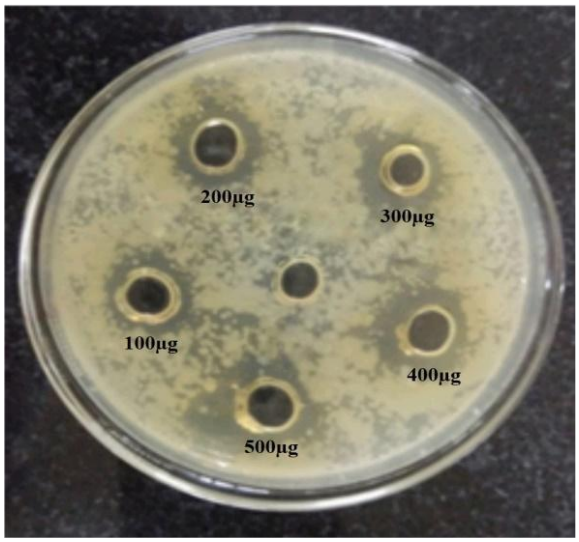


Fig 6: Zone of inhibition for Candida albicans

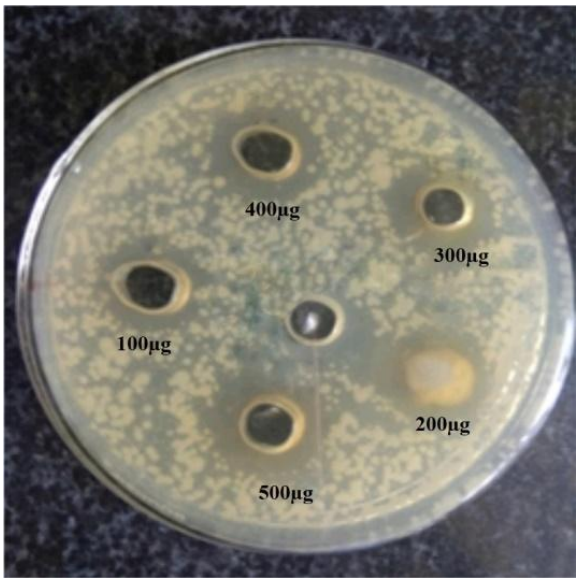


Fig 7: Zone of inhibition for Aspergillus niger

The antimicrobial activity of chloroform extract of leaf of *odina wodier roxb* against tested microbes was shown in Table 1. The results showed the mean zone of inhibition for bacteria ranged were 4.60 ± 0.5 to 9.14 ± 0.5 mm. On the other hand, for *Candidal* strains, the mean zone of inhibition was from 3.2 ± 0.5 to 8.3 ± 0.6 mm. For bacteria, the maximum zone of inhibition (12.6 ± 0.8 mm) was observed against *Staphylococcus aureus.* The minimum mean zone of inhibition (8.92 ± 0.5 mm) was recorded against *Bacillus Subtalis.* In fungi, the maximum zone of inhibition (12.43 ± 0.6 mm) was observed against *C. Albicans,* while the minimum inhibitory zone (8.3 ± 0.5 mm) was shown against Aspergius Niger. The antimicrobial activity of methanolic extracts of leaf of *odina wodier Roxb* was screened against bacterial and fungal strains, and the results are presented in Table 3. The mean zone of inhibition for bacteria ranged between 6.23 ± 0.2 and 14.1 ± 0.2 mm. On the other hand, for inhibition values of fungi were from 5.6 ± 0.2 to 11.26 ± 0.3 mm.

V. DISCUSSION

According to the WHO, medicinal plants would be the best source for obtaining a variety of drugs. This evidence contributes to support and quantify the importance of screening natural products. And were used for the treatment of various diseases. The plants have traditionally provided a source of hope for novel drug compounds as plant herbal mixtures have made a large contribution to human health and wellbeing. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment. The present research work showed the chloroform, ethyl acetate and methanol extracts of *odina wodier Roxb* leaf possess antimicrobial activity against most of the tested pathogens. The result of this study showed that the methanol extracts of *odina wodier Roxb* were more effective than the chloroform extracts demonstrated the highest activity. Among the Gram-positive bacteria and Gram-negative bacteria tested against the leaf extract of *odina wodier, Roxb most sensitive organism was staphylococcus aureus.*

VI. CONCLUSIONS

Finally, it can be concluded that the methanolic extract of odina wodier Roxb had a potential Antimicrobial activity against all the microorganisms tested. Based on this study, isolation and identification of antimicrobial compounds from methanolic extract of odina wodier Roxb will fetch a new natural antimicrobial agent.

VII. STATISTICAL ANALYSIS

The data were expressed as mean ± standard error mean (SEM). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test was followed by Dunnet's test. P values < 0.001 were considered as highly significant, < 0.01were considered as moderately significant and < 0.05 were considered as significant.

REFERENCES

[1] Mathur A, Bhat R, Prasad GBKS, Dua VK, Verma SK and Agarwal PK. "*Antimicrobial activity of plants traditionally used as medicines against some pathogens*". Rasyan J chem.2010; Vol.3, No.4, 615-620.

[2] Valli G, Jeyalakshmi M. "*Preliminary phytochemical and Antioxidant study of Odina wodier leaf extract*." Asian J Phceu Res. 2012;2(4):153-55.

[3] Senthamizhselvi C. "*Traditional knowledge of medicinal plants*." Global Res Analy. 2013;2(12):230-33.

[4] Avinash KR, Jyothi MJ, Ashok kumar CK. Lannea coromendelica: The Researcher's Tree. J Pharma Res. 2011;4(3):577-79.

[5] Prabhakaran M, Sundarapandian SM, Elumala S. "*Phytochemical studies and antibacterial activity of the leaf, bark and root powder of Odina wodier Roxb*". Bioscie Biotech Res Asia. 2010;7(2): 857-77.

[6] Sathish R, Mohd HA, Natarajan K, Lalitha KG. "*Evaluation of wound healing and antimicrobial activity of lannea coromandelica (houtt) merr*". J Pharma Res. 2010; 3(6):1225-28.

[7] Alavijeh PK and Sharma D. "*A study of antimicrobial activity of few medicinal herbs*". Asian J of Plant Sci and Research, 2012; 2 (4):496-502.

[8] Dabur R, Gupta A, Mandal TK, Singh DD, Bajpai V, Gurav AM, Lavekar GS. "*Antimicrobial activity of some Indian medicinal plants*". Afr. J. Trad. CAM (2007) 4 (3): 313 – 318.

[9] Gouranga D, Durbadal O, Bolay B, Monisankar S, Soma G, Suman D. *"Evaluation of antimicrobial potentialities of leaves extract of the plant cassia, tora Linn*". J Phyto. 2010;2(5):64–72.

[10] Premjanu N, Jaynthy C. "*Identification and characterization of the antimicrobial metabolite from an endophytic fungus, Colletotrichum gloeosporioides isolated from Lannea corammendalica*". Int J ChemTech Res. 2014-15;7(01):369-74.

[11] Lohithasu D, Hema ND, Ramana KV, Murthy. "*Physico-chemical and microbial studies of lannea gum*". Int Res J Pharma. 2017; 8 (4): 50-58.

[12] Ayyappan, S.R., R. Srikumar and R. Thangaraj. Int J of Microbiology Rese, (2010);1(2), 67-71.