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IN-VITRO ANTIMICROBIAL ACTIVITY OF *CROTALARIA VERRUCOSA* MEDICINAL PLANT

J. Sirajudeen^{1*}, R. Elamparithi¹, A. K. Umera Begam²

^{*1 & 1} PG and Research Department of Chemistry, Jamal Mohamed College, (Autonomous)

Tiruchirappalli, Tamil Nadu, India.

²PG Department of Bio Chemistry, Holy Cross College, (Autonomous)

Tiruchirappalli, Tamil Nadu, India.

ABSTRACT

Communicable diseases are very serious problem at present as well as in past also in developing in addition to developed countries. It is caused by various pathogenic microbes like fungi, bacteria, parasites and virus etc. In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, a greater attention has been paid to antimicrobial activity screening and evaluating methods. Herbal medicines are generally used for healthcare because they have low price and wealthy source of antimicrobial properties. Antimicrobial activity of various plant parts such as bark, stem, leaf, fruit and seed against Gram negative, Gram-positive bacteria and fungal species, using hydroalcohol extraction by well diffusion method. The microorganisms employed were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium* sp. and *Aspergillus terreus*. Hydroalcoholic extract of *crotalaria verrucosa* were potentially efficient with variable efficiency against the tested bacterial and fungal strains at different concentrations.

KEYWORDS

Antimicrobial activity, Secondary metabolites and Pharmaceuticals.

Author for Correspondence:

Sirajudeen J,
PG and Research Department of Chemistry,
Jamal Mohamed College, (Autonomous),
Tiruchirappalli - 20, Tamil Nadu, India.

Email: sirajudeen2003@gmail.com

Available online: www.uptodateresearchpublication.com

INTRODUCTON

Plants are nature's gift to human which in the form of food, oxygen, in addition to shelter. From ancient periods, they have served as defence mechanism used by our ancestor to struggle against diseases. Also it has valuable source of natural products for maintaining human health going together with intensive studies for natural therapies. According to the information of world health organization (WHO), about 80% of the humanity relies on

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traditional herbal therapies. Most traditional drugs administered today were derived from plant life. As a consequence, they produce metabolites that could have some biological activity (e.g. antioxidant, antimicrobial), which could be a biotechnological potential target in the health and agricultural sectors^{1,2}.

Globally, the use of botanicals as alternatives to intake of synthetic drugs has increased yearly. Botanicals have several advantages over synthetic antibiotics, such as little or no harmful effect in humans or on the environment, and cost efficiency³. However, botanical pesticides have some limitations, such as slow and lower efficacy compared with chemical drugs and fewer efficacies thus, the growth of novel formulations with enhanced efficacy and longer time of products to store is required⁴⁻⁷. Although there are various medications available for treatment of various diseases but they have exhibited numerous undesired side effects related with their uses, so other efficient pharmacological alternatives as natural products from medicinal plants have been suggested. Now days, the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries to cope increasing number of infectious agents, becoming resistant to commercial antimicrobial drugs.

In recent years, several resistances in human pathogenic microbes have developed due to the random use of commercial antimicrobial drugs frequently employed in the management of infectious disorders. The evaluation of plant extracts and plant products for antimicrobial activity has shown a potential source of new anti-infective agents. Medicinal plants characterize a rich source of antimicrobial agents and basis of new drugs which are tremendously useful for synthetic modification of optimal of biological activity⁸.

Crotalaria verrucosa L. (Fabaceae) commonly called as blue rattlesnake which is distributed inside India, in the tropical regions, from Himalayas to Ceylon. It is much branched herbaceous, usually annual plant with blue, occasionally white flowers. The fruit is a small globose yellow to orange berry. The leaves 5 -15cm long are ovate rhomboid and

obtuse. The leaf extract is applied to soothe skin allergies⁹. Leaves extract is used in impetigo and scabies both outwardly and internally, also considered effective in declining salivation and also the leaf extract is given vocally to cure jaundice. Both aqueous and ethanolic extracts of aerial parts of *C. verrucosa* were efficient for fertility and estrogenic implantation in Albino rats and also it has substantial hepato protective property against paracetamol induced hepatotoxicity study models in Wistar rats^{8,10}. The present investigation was carried out on leaves of *Crotalaria verrucosa* L. in order to determine the anti-bacterial and antifungal efficacy of hydroalcoholic extract.

MATERIAL AND METHODS

Determination of Antibacterial activity

Pathogens

For this study, both Gram Positive (*S. aureus* (MTCC 1430) and *Bacillus cereus* (MTCC 143)) and Gram Negative (*Escherichia coli* (MTCC 433), and *Proteus mirabilis* (MTCC 425)) bacteria were used to determine the antibacterial activity.

Antibiotic Susceptibility Testing

Bacterial broth was prepared by dissolving 1.3 g of nutrient broth (NB) in 100 ml of distilled water. Then, took a loopful of bacterial culture from the slant and inoculate bacteria in the broth medium. Then, incubate the culture broth for 18 - 24 hrs at 37°C. The antibacterial activity was carried out by using hydroalcohol leaves extract of *Crotalaria verrucosa* L. by agar well method^{11,12}. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 24 hrs old broth culture of respective bacteria. Consequently, using sterile borer, well of 0.5 cm diameter was made into the each agar plate and then 50, 100 and 150µl containing 500µg/ml concentration of hydroalcohol extract in aseptic condition filled into the respective wells. Then it was placed at room temperature for an hour and then transfers the plates for incubation about 24 hrs at 37 °C. The results were recorded by measuring the diameter of zone of inhibition using a transparent meter rule. Tetracycline is the standard drugs for antibacterial activities, respectively.

Determination of antifungal activity

Preparation of culture inoculums

The stock cultures of fungi (*Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Penicillium* sp. and *A. terreus*) used in this study was maintained on potato dextrose agar slants at 4°C. Inoculums was prepared by suspending a loop full of fungal cultures into 10 ml of potato dextrose broth and was incubated at 28°C ± 2°C for 48 hours.

Agar well-diffusion method

Agar well-diffusion method was followed to determine the antifungal activity. Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 24 hours culture and 48 hours old - broth culture of respective bacteria and fungi. Agar wells (5mm diameter) were made in each of these plates using sterile cork borer. About Dms (Dimethyl sulfide), Positive control (Ketoconazole), 30, 60 and 90µl of different solvent plant extract powder were added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions The plates were incubated in an upright position at 28°C ± 2°C for 48hours fungi. Results were recorded, as the presence or absence of inhibition zone. The inhibitory zone of the zones was measured using diameter measurement scale. The effect of plant extract was compared with standard antibiotics. Triplicates were maintained and the average values were recorded for antifungal activity.

RESULTS AND DISCUSSION

The capacity of the plant extract to kill or hinder the growth of pathogenic microbes viz., *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis* and *Salmonella typhi* and *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Penicillium* sp. and *A. terreus* were evaluated by *in-vitro* conditions and the results are presented in Table No.1 and 2. The hydro alcoholic leaves extract of *C. verrucosa* was used for their antibacterial activity by determining the zone of inhibition (ZOI) with the help of scale against tested organisms by agar well diffusion method for the estimation of potency of the antimicrobial

substances. Extracts obtained from the leaves of *Crotalaria verrucosa* L. using a hydro alcohol solvent by successive extraction method were tested against human pathogenic Bacteria and Fungi; viz., *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis* and *Salmonella typhi* and *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Penicillium* sp. and *A. terreus*.

The hydro alcoholic leaves extract of *C. verrucosa* showed antibacterial activity 15 mm towards *E. coli*, 10 mm showed lowest inhibition activity against both *Proteus mirabilis* and *Salmonella typhi* and the same showed maximum inhibition (20 mm) against *S. aureus* (Table No.1). The hydro alcoholic leaves extract of *C. verrucosa* showed antifungicidal activity against *Aspergillus flavus* and *A. terreus* with 12 mm inhibition which is highest inhibition activity compared with other organisms such as *A. fumigates* and *Penicillium* were showed moderate activity (11 mm and 10 mm). The *C. verrucosa* leaves extract showed lowest inhibition activity against *A. niger* (7 mm) (Table No.2).

Earlier studies on phytochemical screening were investigated on the ethanolic extract of *Crotalaria verrucosa* L. confirmed the presence of flavonoids and phytosterols in this plant¹³⁻¹⁶. The presence of bioactive compound in the plant *C. verrucosa* such as Coumarins, flavonoids, glycosides, phenols, saponins, steroids and tannins which may be the responsible for the observed antimicrobial property. Commonly, the bacterial infections are main cause of morbidity and death. New therapeutic treatment can be improved constantly increasing with the advances of healing properties in medical science. From plant source it has much interest and efforts to determine bioactive compounds that can achieve as accurate antimicrobials agent to alter the synthetic ones^{17,18}. In most of the diseases in human, the microbes do not involve directly but it allows the bacteria to beat the natural defensive mechanisms of the body during the damage of the skin, which leads to infections¹⁹. It is mandatory to inspect and authenticate various indigenous drugs along with better understanding of their biological and pharmacological properties^{18,20}.

Many antimicrobial compounds have been effectively acted against several diseases which can be derived from medicinal plant as phytochemicals which can serve as a prototype to develop less lethal and efficient drug in controlling the development of microbes^{17,18}. Nowadays, most of the people interested to use drugs with safe, efficient from plant based products that are alternative to the profitable synthetic medicine. These Secondary metabolites are act as a protective mechanism against microorganisms, insects and other herbivores²¹ and these antimicrobial drugs have vast potentially therapeutic value²².

Numerous reports are available on the antibacterial efficiency of leaf, root and bark extracts of *C. verrucosa*. Prabhakar et al, 2015⁸ revealed that the seed extracts of *C. verrucosa* and *D. erecta* are potentially antibacterial against *B. subtilis*. Only the methanolic extract of *C. verrucosa* was effective against *P. aeruginosa*, *S. aureus* growth the methanolic, ethanolic and acetone extracts of *C. verrucosa* shown inhibitory effect. In our study, the hydro alcoholic extract has significant effect against some bacteria and fungi.

Table No.1: Effect of antibacterial activity of hydro alcohol leaves extract of *Crotalaria verrucosa* L. against fungi

Zone of inhibition (mm)						
S.No	Name of the fungi	DMSO	Positive control (Tetracycline)	50µl	100µl	150µl
1	<i>Escherichia coli</i>	-	14.0±5.29	7.05±0.17	10.5±0.13	15.8±0.21
2	<i>Proteus mirabilis</i>	-	13.0±0.21	3.03±0.16	5.02±0.12	10.04±0.10
3	<i>Salmonella typhi</i>	-	15.3±0.57	6.04±0.09	8.2±0.15	10.6±0.10
4	<i>S. aureus</i>	-	17.6±2.51	10.05±0.08	13.06±0.05	20.4±0.13

Table No.2: Effect of antifungal activity of hydro alcohol leaves extract of *Crotalaria verrucosa* L. against fungi

Zone of inhibition (mm)						
S.No	Name of the fungi	DMSO	Positive control (Ketoconazole)	30µl	60µl	90µl
1	<i>Aspergillus flavus</i>	-	-	9.05±0.17	11.5±0.19	12.8±0.21
2	<i>A. niger</i>	-	-	6.03±0.16	7.02±0.18	7.04±0.20
3	<i>A. fumigatus</i>	-	-	8.04±0.09	10.2±0.10	11.6±0.12
4	<i>Penicillium sp.</i>	-	-	7.05±0.08	8.06±0.11	10.4±0.17
5	<i>A. terreus</i>	-	-	10.6±0.06	11.8±0.08	12.5±0.10

CONCLUSION

From the above results we can bring to a close that plant extract has remarkable antimicrobial activity. We recognize that microbes are gaining resistance day by day towards the antibiotics drugs, so that some natural creation should be try to defeat these antibiotic resistant organisms. The hydro alcoholic extract leaves of *C. verrucosa* were more effective against the selected bacteria and fungi. Thus, the present work ascertains the value of plant used in pharmacy, which could be of significant interest to the growth of new drugs.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Martinez J L. Antibiotics and antibiotic resistance genes in natural environments, *Science*, 321(5887), 2008, 365-367.
2. Monk B C, Goffeau A. Outwitting multidrug resistance to antifungals, *Science*, 321(5887), 2008, 367-369.
3. El-Wakeil N E. Botanical pesticides and their mode of action, *Gesunde Pflanz*, 65(4), 2013, 125-149.
4. Gurjar M S, Ali S, Akhtar M, Singh K S. Efficacy of plant extracts in plant disease management, *Agric Sci*, 3(3), 2012, 425-433.
5. Bhagat S, Birah A, Kumar R, Yadav M S, Chattopadhyay C. Plant disease management: prospects of pesticides of plant origin, In: Singh D, editor, *Advances in Plant Biopesticides. India: Springer*, 2014, 119-129.
6. Marutescu L, Popa M, Saviuc C, Lazar V, Chifiriuc M C. 8 - Botanical pesticides with virucidal, bactericidal, and fungicidal activity. In: Grumezescu AM, editor. *New pesticides and soil sensors, Oxford, UK: Academic Press*, 2017, 311-335.
7. Vu T T, Kim H, Tran V K, Vu H D, Hoang T X, Han J W, et al. Antibacterial activity of tannins isolated from *Sapium baccatum* extract and use for control of tomato bacterial wilt, *PLoS ONE*, 12(7), 2017, 1-12.
8. Prabhakar G, Kamalakar P, Ashok vardhan T, Shailaja K. *In-vitro* screening of antibacterial activity of seeds of *crotalaria verrucosa* L. and *Duranta erecta* L, *European journal of pharmaceutical and medical research*, 2(4), 2015, 411-419.
9. Sirajudeen J and Elamparithi R. Preliminary Phytochemical Analysis of *Crotalaria verrucosa* Leaves, *Asian journal of research in chemistry and pharmaceutical sciences*, 7(1), 2019, 278-281.
10. Ramesh Kannan N, Agnel Arul John N and Natarajan E. Pharmacognostical, physico-chemical, phytochemical analysis and anticancer screening of *Crotalaria verrucosa* L, *World journal of pharmacy and pharmaceutical sciences*, 2018, 7(6), 998-1013.
11. Sinclair J B and Dhingra O D. *Basic Plant Pathology Methods*, CRS Press, Inc. Boca Raton, Florida, 2nd Edition, 448, 1995.
12. Ahmad I Z, Mehmood and Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties, *J Ethnopharmacol*, 62(2), 1998, 183-193.
13. Nawrin K, Billah M M, Javed M S U, Roy A, Ahmed A K M R and Islam M N. Antipyretic, Antidiabetic, Thrombolytic and CNS Depressant Potential of Ethanol Extract of *Crotalaria verrucosa* L. Leaves, *American Journal of Biomedical Sciences*, 7(4), 2015, 198-204.
14. Riazunnisa K, Prasad M V, Sudha G S, Khadri C H. *In-vitro* antibacterial activity and phytochemical studies of leaf extracts of *Adhatoda vasica* and *Crotalaria verrucosa*, *World Journal of Pharmacy and Pharmaceutical Studies*, 4(6), 2015, 509-511.
15. Singh U, Jialal I. Oxidative stress and atherosclerosis, *Pathophysiology*, 13(3), 2006, 129-142.
16. Lekharani C, Yanadaiah J P, Ravindra R K, Lakshman K D, Venkatasubbaiah M. Hepatoprotective activity of aqueous ethanolic extract of aerial parts of *Crotalaria verrucosa* Linn, Paracetamol-induced hepatotoxicity in rats, *Journal of Pharmaceutical and Biological Sciences*, 1(4), 2013, 50-55.
17. Antara Sen and Amla Batra. Evaluation of Antimicrobial Activity of Different Solvent Extracts of Medicinal Plant: *Melia azedarach* (L.), *International Journal of Current Pharmaceutical Research*, 4(2), 2012, 67-73.
18. Kaleeswaran B, Ramadevi S. Phytochemical analysis and pathogenic inhibition activity of *Pedalium murex* (L.) against Urinary Tract Infection Bacteria, *International*

Journal of Current Research, 8(09), 2016, 38546-38551.

19. Rahat Ejaz, Usman A. Ashfaq, Sobia Idrees. Antimicrobial potential of Pakistani medicinal plants against multi-drug resistance *Staphylococcus aureus*, *Journal of Coastal Life Medicine*, 2(9), 2014, 714-720.
20. Kuldeep dharma, Ruchi Tiwari, Sandip Chakraborty, Mani Saminathan, Amit Kumar, Karthik K, Mobd. Yaqoob Wani, Amarpal, Shoor Vir Singh and Anu Rahal. Evidence Based Antibacterial Potentials of Medicinal Plants and Herbs Countering Bacterial Pathogens Especially in the Era of Emerging Drug Resistance: An Integrated Update, *International Journal of Pharmacology*, 10(1), 2014, 1-43.
21. Zablutowicz R M, Hoagland R E and Wagner S C. Effect of saponins on the growth and activity of rhizosphere bacteria, *Adv Exp Med Biol*, 405, 1996, 83-95.
22. Aiyegoro O A and Okoh A I. Use of bioactive plant products in combination with standard antibiotics: Implications in antimicrobial chemotherapy, *Journal of Medicinal Plants Research*, 3(13), 2009, 1147-1152.

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