Advances in Bioresearch
Adv. Biores., Vol 15 (5) September 2024: 350-353
©2024 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.5.350353

Advances in Bioresearch

SHORT COMMUNICATION

Evaluation of Antimicrobial Potential of *Entada rheedei* Seed *Extract*

M. B. Kawade[1,3], S. V. Rupanar[3], S. P. Rasale[1], S. R. Katade[2*]
[1]Prof. Ramkrishna More Arts, Commerce & Science College, Akurdi, Pune (India)
[2]Modern Arts, Commerce & Science College, Ganeshkhind, Pune (India)
[3]Ajeenkya D. Y. Patil School of Engineering, Pune (India)
Corresponding Author: Sushma R. Katade
Email: sushmarkatade@gmail.com

ABSTRACT
Entada rheedei is a large woody climber growing naturally throughout tropical Africa, South asia and India. This plant is commonly known as African dream herb & it is one of the medicinal plants which contain biological active compounds like saponins, flavonoids and terpenoids. The ethnopharmacological uses of Entada rheedei is known in many countries. Now day's scientists have a great interest towards the medicinal plant having biological potential. In modern system of herbal medicine, drug standardization is essential in order to assess quality and purity of herbal drug. Evaluation of biological potential of medicinal plant is one of the important steps in standardization of drug. The synthesis of potent antimicrobial reagents is needed. The main objective of the study is to evaluate the antimicrobial potential of seed of Entada rheedei. The present study reports study of antimicrobial activity of Entada rheedei seed extract against various microbial strains. The antimicrobial activity of seed extract of Entada rheedei have been checked in terms of MIC by using microorganisms like Staphylococcus aureus (NCIM 2178), Bacillus subtilis (NCIM 2063), Proteus mirabilis (NCIM 2388), Candida albicans (NCIM 3100), E. coli (NCIM 2065) and Aspergillus niger (ATCC 504) by disc diffusion method. Hexane, ethyl acetate and methanol seed extract of Entada rheedei actively inhibited the growth of Staphylococcus aureus and Proteus mirabilis whereas, it is moderately inhibited the growth of Bacillus subtilis with MIC 0.5 to 1 µl/ml. The methanol extract effectively inhibited the growth Candida albicans and Aspergillus niger as compared to other extracts. This study revealed the antimicrobial potential of seed of Entada rheedei may be due to bioactive compounds.
Keywords: *Antimicrobial activity, disc diffusion assay, Entada rheedei seed, Disc diffusion method.*
Abbreviations: *MIC – Minimum Inhibitory Concentration, NCIM - National Collection of Industrial Microorganism, ATCC - American Type Culture Collection, E. coli – Escherichia coli.*

Received 24.05.2024 Revised 21.06.2024 Accepted 11.08.2024

How to cite this article:
M. B. Kawade, S. V. Rupanar, S. P. Rasale, S. R. Katade. Evaluation of Antimicrobial Potential of *Entada rheedei* Seed Extract. Adv. Biores., Vol 15 (5) September 2024: 350-353.

INTRODUCTION

Plant derived medicines have made large contribution to human health and wellbeing [5]. Even though pharmacological industries have produced many new antibiotics, microorganisms developed resistance to most of these antibiotics which is a global concern [1]. The genus *Entada* consist of 30 species of trees, shrubs and tropical lianas. Out of these, 21 species are known from Africa, six from Asia, two from American tropics and one with pantropical distribution [10]. *Entada rheedei* is a large woody liana or climber growing naturally throughout tropical Africa and South Asia [3]. The new location of *Entada rheedei* has also been identified in India i.e. in Sangli and Pune district (Maharashtra state) [2,3]. *Entada rheedei* has various traditional medicinal uses including the treatment of jaundice, diarrhea, musculoskeletal problems [7, 9]. Tobacco made from the seeds of *Entada rheedei* has been reported to cause vivid dreaming. So, this plant is commonly known as African dream herb [3]. By considering the medicinal importance of *Entada rheedei*, the present research work has been carried out to determine the antimicrobial activity of different solvent extracts of seed kernel of *Entada rheedei* against bacterial species like gram +ve and gm -ve bacteria and fungi through the disc diffusion assay. Several studies have been confirmed the antimicrobial activity of different *Entada* species. However, there is an insufficient

information regarding antimicrobial activity of *Entada rheedei* . This study revealed the antimicrobial activity of seed extracts of *Entada rheedei* as a part of exploration for new and novel bioactive compounds.

MATERIAL AND METHODS

Dried seeds of *Entada rheedei* was collected from the forest of Bhimashankar, district Pune (Maharashtra), India. The plant was identified and authenticated by Botanical Survey of India, Pune.

Preparation of extract

The extracts of powdered seed kernel of *Entada rheedei* were prepared by Soxhlet extraction by using nonpolar to polar system (Successive extraction by hexane, ethyl acetate and methanol). After reflux, the Soxhlet flask was cooled at room temperature and extract was collected in the round bottomed flask of Rotary evaporator. By using rotary evaporator, the solvent is recovered and the crude extract was collected.

Culture used for antimicrobial activity

The microorganisms used were as follows: *Staphyllococcus aureus* NCIM2178, *Bacillus subtilis* NCIM2063, *P. mirabilis* NCIM2388, *Candida albicans* NCIM3100, *E.coli* NCIM2065, *Aspergillus niger* ATCC504.

Culture medium

The bacterial isolates were first subcultured in nutrient broth and incubated at 37^0c for 18 hours whereas fungal isolates were subcultured on Sabouraud Dextrose Agar (SDA) at 25^0c for 72 hours. The composition of nutrient broth and SDA is shown in table 1 and table 2 respectively.

Composition of nutrient broth

Ingredients	g/L
Peptones	10 g
Beef extract	1 g
Yeast extract	2 g
Sodium Chloride	5 g

Composition of SDA

Ingredients	g/L
Mycological peptone (enzymatic digest of casein and animal tissues)	10 g
Dextrose	40 g
Agar	15 g

Antimicrobial activity

Antimicrobial activity test was performed by the commonly used Agar diffusion method, which was designed to determine the smallest amount of the antibiotic needed to inhibit the growth of microorganism. Sterile cotton swabs were taken and dipped it into a culture of Test organism suspension. The entire agar surface of each plate was inoculated first in horizontal and then in vertical direction to ensure the even distribution of microorganism over the agar surface using the swab. The agar surface is allowed to dry for 5 minutes. A cork borer was sterilized by autoclaving it. The Mueller –Hinton agar plates were obtained and aseptically punched (4 mm) holes in the agar using a cork borer. Using a wax pencil, the underside of the petri was marked to label the wells. The test solution was added with the help of micropipette in the well. This procedure was repeated for all wells. All plates were incubated at 37^0 c for 24 to 48 hours in an incubator.

RESULT

All plates were examined for the clear zone of inhibition surrounding the discs. The diameter of zone of inhibition was measured in mm using a ruler on the underside of the plate and zone size is recorded which is shown in Table 1. The MIC values of seed extracts for different microorganisms are shown in Table 2. The concentration used for the study is 1µl/ml. The Antibiotic used for Bacteria is Streptomycin 10ug/ml and for fungi is Flucanazole, 10ug/m**l.** Methanol extracts was found to be effective against bacteria *E. coli*, *Staphylococcus aureus*, *Proteus mirabilis* and moderate against *Bacillus subtilis*. It was also showed strong antifungal activity against *Candida albicans* and *Aspergillus niger*. Hexane extract showed strong antimicrobial activity against *Bacillus subtilis*. Ethyl acetate extract showed very effective antimicrobial activity against *Staphylococcus aureus* and *Proteus mirabilis* and moderately inhibited the growth of *Aspergillus niger*.

DISCUSSION

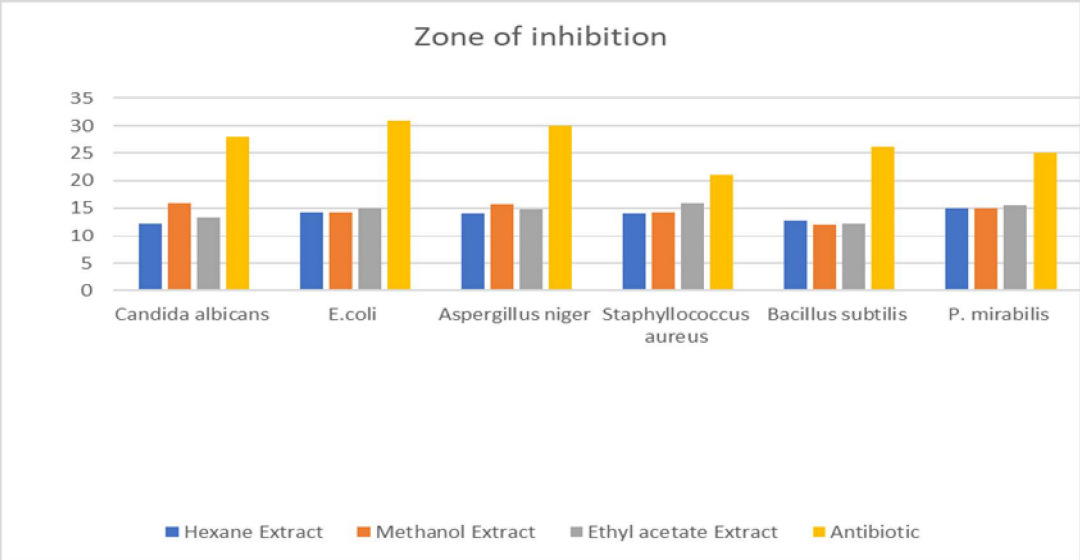
This study revealed that *E. coli* and *Staphylococcus aureus* were highly susceptible to methanol extract of seed extract of *Entada rheedei*. The methanol extract was also found to be an effective antifungal agent against *Candida albicans* and *Aspergillus niger*. The *E. coli* can cause Urinary tract infection [11] and *Staphylococcus aureus* can cause bone and joint infections [4]. The fungi like *Candida albicans* and *Aspergillus niger* also causes serious life-threatening infections like lung infections, weak immune system, asthma and sinus problem [6, 8]. The methanol extract of seed kernel of *Entada rheedei* can reduce the infections caused by these bacteria and fungi. Hexane extract also reduced the growth of *Bacillus subtilis* effectively and showed moderate antimicrobial activity against *Candida albicans*. Ethyl acetate extracts also actively inhibited the growth of gram-positive and gram-negative bacteria like *Staphylococcus aureus* and *Proteus mirabilis* respectively. It has moderately inhibited the growth of *Aspergillus niger* and *Bacillus subtilis*.

Table 1. Zone of Inhibition (mm)

Sr. No.	Name of Microorganism	Hexane extract	Mean	Ethyl acetate extract	Mean	Methanol extract	Mean	Antibiotic
1	*Candida albicans*	12, 12, 12, 13,12	12.2	13, 14, 13, 14, 13	13,4	16, 15, 16, 17, 15	15.8	28
2	*E. coli*	14, 13, 14, 17, 13	14.2	13, 16, 14, 17, 15	15	14, 15, 14, 14, 14	14.2	31
3	*Aspergillus niger*	15, 15, 15, 13, 12	14	13, 15, 14, 17, 15	14.8	16, 15, 16, 16, 15	15.6	30
4	*Staphylococcus aureus*	14, 13, 14, 15, 14	14	17, 16, 15, 15, 16	15.8	13, 14, 14, 15, 15	14.2	21
5	*Bacillus subtilis*	12, 13, 14, 12, 13	12.8	11, 12, 13, 12, 13	12.2	13, 12, 11, 12, 12	12	26
6	*Proteus mirabilis*	16, 14, 14, 16, 15	15	16, 13, 16, 16, 16	15.4	15, 15, 15, 15, 151	15	25

Table 2. MIC values in µl/ml

Sr. No.	Extract	Microbial strains					
		Candida albicans	***E.coli***	***Aspergillus niger***	***Staphylococcus aureus***	***Bacillus subtilis***	***Proteus mirabilis***
1	Hexane Extract	-	1	1	1	-	0.5
2	Methanol Extract	0.5	1	0.5	1	-	0.5
3	Ethyl Acetate Extract	-	0.5	0.5	0.5	-	0.5



Graph1: of Zone of inhibition shown by microorganisms

 © 2024 Author

CONCLUSION

The antibacterial and antifungal activity of extracts of seed kernel of *Entada rheedei* is may be due to the presence of active antimicrobial compounds in the seed kernel of this plant. The antimicrobial compounds from the plant *Entada rheedei* have not been reported till date. Therefore, standardization and detailed phytochemical exploration of this plant is needed for the development of potent antimicrobial drug. The further phytochemical investigation is needed for the conservation, cultivation and commercialization of this endangered plant.

ACKNOWLEDGEMENT

Authors would like to thank to Principal, Modern Arts, Commerce & Science College, colleagues for their assistance & support in this research.

REFERENCES

1. Bakht, J., Noor, N., Iqbal, A. and Shafi, M. (2020). Antimicrobial activity of different solvent extracted samples from the leaves and fruits of Capsicum annuum. Pak J Pharm Sci, *33*(1):27-32.
2. Bhise, M. R., Rahangdale, S. S., Rahangdale, S.R. and Kambhar, S.V. (2013). Floristic study of kalbhairavanatha sacred grove, Terungan, Ambegaon Taluka, Pune. Res & Reviews: J Bot. 2(1):17-24.
3. Deokar, R. R., Mane, S. R., Kamble, S. R., Patil, S. R. (2017). A New Locality Record for An Endangered Plant Species Entada Rheedei Sprong (Mimosaceae) In Sangli District, Maharashtra. World Journal of Pharma Res. 6(17), pp.405-409.
4. Dohin, B., Yves G., Kohler, R., Gérard L., Vandenesch, F., Vanhems, P., Floret, D., Etienne J. (2007). Pediatric bone and joint infections caused by Panton-Valentine leukocidin-positive Staphylococcus aureus. The Pediatric infectious disease journal. 26(11): 1042-1048.
5. Igbinosa, O. O., Igbinosa, E. O., Aiyegoro, O. A. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropha curcas (Linn). African journal of pharma and Pharmacol. 3(2):058-062.
6. Nobile, C. J., Johnson, A. D. (2015) Candida albicans biofilms and human disease. Annual review of microbial. 69:71-92.
7. Nzowa, L.K., Teponno, R.B., Tapondjou, L.A., Verotta, L., Liao, Z., Graham, D., Zink, M.C. and Barboni, L., 2013. Two new tryptophan derivatives from the seed kernels of Entada rheedei: effects on cell viability and HIV infectivity. *Fitoterapia*, *87*, pp.37-42.
8. Schuster, E., Dunn-Coleman, N., Frisvad, J. C., Van Dijck, P.W. (2002). On the safety of Aspergillus niger–a review. Applied microbial. and biotechnol. 59:426-435.
9. Sugimoto, S., Matsunami, K., Otsuka, H. (2012). Medicinal plants of Thailand. II: chemical studies on the seed kernels of Entada rheedei Sprengel. Journal of natural medicines, 66: 552-557.
10. Tateishi, Y., Wakita, N., Kajita, T. (2008). Taxonomic revision of the genus Entada (Leguminosae) in the Ryukyu Islands, Japan. Acta Phytotaxonomica et Geobotanica, 59(3):194-210.
11. Vincent, C., Boerlin, P., Daignault, D., Dozois, C. M., Dutil, L., Galanakis, C., Reid-Smith, R. J., Tellier, P. P., Tellis, P. A., Ziebell, K., Manges, A.R. (2010). Food reservoir for Escherichia coli causing urinary tract infections.

Copyright: © 2024 Author. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.