Original Research Paper Botany

PHYTOCHEMICAL EVALUATION AND ANTIMICROBIAL EFFICACY OF *Entada rheedii* Spreng.

Arulkumar M	PG Department of Botany, AA Government Arts College, Namakkal, Tamil Nadu, India
Velmurugan C	PG Department of Botany, AA Government Arts College, Namakkal, Tamil Nadu, India
E.G. Wesely	PG Department of Botany, AA Government Arts College, Namakkal, Tamil Nadu, India
Nazneen Bobby	MD, Vignan university, VFSTR, Vadlamudi post, Guntur – 522 213, Andhra Pradesh, India
Nathan Vinod Kumar	National Institute of Oceanography-CSIR, Regional Centre, Cochin-682018, Kerala, India

ABSTRACT *Entada rheedii Spreng., is a woody climber shrub of the Family Fabaceae which is used in many traditional medicines. It is rich in many phytochemicals. The seeds areused as narcotic, emetic, febrifuge, alexiteric andantiperiodic. However, the bio potential of some parts was not explored. In the present work, we investigate the preliminary phytochemicals of leaf extract prepared using various solvents.The antimicrobial efficacy of these extract against selected microorganisms namely against E.coli, Pseudomonas aeruginosa, Bacillus subtilis and Salmonella typhi were evaluated. The solvent extracts were found very significantly inhibiting Pseudomonas at the highest concentrations very effectively. These extracts were efficiently inhibiting the multidrug resistant pathogenic strains. The phytochemical analysis revealed that the extract contained alkaloids, tannins, terpenoids etc. which may be attributed to the antimicrobial effect of the extract. The leaves of the Entada rheediiwas less explored for its bio-potential and hence the work is a preliminary report with significant findings of its bio-potential.*

KEYWORDS : *Entada rheedii*,antimicrobial, leaves, multidrug resistance, phytochemicals

INTRODUCTION

The phytomedicines are part of our culture and were followed as traditional or indigenous knowledge practices. However, the modern medicines replaced them from our lifestyle. Though there are tremendous development in the field of modern medicines, laterally there are many new challenges arising. Among them, a major challenge is antibiotic resistance mechanism in many pathogens. The fact is that the antibiotics become insufficient for the curing of the microbial associated infections and diseases. Over the time, the demand for the herbal based formulation for curing these diseases are increasing. India being a country with rich bioresource has plenty of herbal formulations and many plants as well astrees parts were widely used for curing many diseases in different forms.

Entada rheedii Spreng., is a woody climber shrubof the Family Fabaceae inhabitant to most tropical countries including India **[1,2].**It is known as Pramior African dream herb and widely used to cure Body pain, musculo-skeletal problems.The seeds areused by the folkloric medicinal practitioners, locally known as narcotic, emetic, febrifuge, alexiteric andantiperiodic. Triterpenes isolated from seed of *E. rheedii* has antiproliferative and antioxidant activity**[3,4].** Infusion of E. rheediibark were used in Tanzania to cure scabies [5].Similarly, pains and itch are mitigated by bark and seeds of E. rheedii in South-East Asia **[5]**. Phytochemical investigation of E. rheedii has not yet done extensively. Recently, some reported on the isolation of flavonoids from the bark of *E. rheedii* Spreng**[6]** .Moreover, seed kernels ofE. rheediihavebeen reported to contain tryptophan derivatives, triterpenoidsaponins (rheediinoside A and B) **[3]**,tryptorheedei A and tryptorheedei B **[7]**,oleanane-type triterpene oligoglycosides (rheedeiosides A,B, C and D), thioamide glycoside andcis-entadamide A-β-D-glucopyranoside**[8]**.

The demand for phyto-medicines is increasing in the recent days. Hence, there are new avenues opening in identifying new plant based drugs or formulations to cure many disorders. This research paper focuses on the preliminary study of Phytochemistry of leave extract of *E. rheedii* and to evaluate the antimicrobial activity against selected pathogens.

Materials and Methods

Collection of Plant Material

Leaf samples of Entada rheediiSpreng. were collected from Namakkal District of Tamil Nadu, India. The samples were shade dried and powdered for extraction of phytochemicals.

Figure 1: Habitat of *Entada rheedii* Spreng.

Preparation of leaf extract

E. rheedii leaves was dried, powdered and 10g of the powder was subjected to Soxhlet extraction. Chloroform, methanol, ethanol and isopropanol were used in the order of polarity. The extraction was performed overnight to obtain concentrated aqueous extract of the leaves. Further the extract was concentrated using a rotary evaporator and was stored at 4°C for further use.

Phytochemical Analysis of Extract

Phytochemical evaluation of the leaf extract of *E. rheedii* was

performed using the standard procedures. Different phytochemicals, namelyflavonoids, saponins, steroids, terpenoids, tannins, carbohydrates and glycosides were screened **[9].**

Agar diffusion assay

The modified agar well diffusion method was employed. 24hrs old bacterial cultures of *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Salmonella typhi* were inoculated onto Muller Hinton agar media (HiMedia, Mumbai) by spread plate technique. 6mm diameter wells were made using sterile gel puncher. Various extracts of *E. rheediileaves* was dissolved in DMSO (Dimethyl Sulfoxide) from which 25, 50 75 and 100µl of extract were added into agar wells. The plates were sealed and incubated at 32 ± 2 oC for 24 hr. The inhibition zone diameter was recorded after the incubation period. All the experiments were conducted in triplicates**[10].**

Phytochemicals	Extracts			
	Chloroform	Ethanol	Methanol	Isopropanol
Flavonoids	+	-	-	-
Saponins	-	-	-	-
Steroids	-	-	-	-
Terpenoids	+	+	+	+
Tannins	-	+	+	+
Carbohydrates	-	-	-	-
Glycosides	-	-	+	-

Table 1. Phytochemical analysis of leaf extract of *E. rheedii*

Results and Discussion

Phytochemical Analysis of *E. rheedii* Leaf Extract

Phytochemical analysis of the *E. rheedii* leaf extract prepared using various solvents were performed (Table 1). The results revealed the presence of terpenoids in all the extracts. Saponin, steroids and carbohydrates were not detected in any extracts. Tannins and terpenoid content were extracted using ethanol, methanol and isopropanol. In some previous works, phytochemistry of other species of *Entada* were reported.Phytochemical study of ethanol extract of seed of *E. scandens* were investigated which revealed the presence of alkaloids, glycosides, tannins, flavonoids and saponins[11].However, this is the pioneering report on the phytochemical analysis of leaf extract of *E. rheedii*.

Antimicrobial Activity of Leaf Extractusing Well Diffusion Method

Antimicrobial activity of *E. rheediileaf* extract was evaluated using well diffusion assay. Pathogenic cultures namely *E.coli, Bacillus subtilis, Salmonella typhi and Pseudomonas aeruginosa* were used in the experiment. The spread plated Muller Hinton media were punched and added 2 different concentrations of aqueous bark extract and incubated for 24 hrs. The zone of inhibition was measured following incubation time (Table 2).

The multidrug resistant isolates were used for the study and there was a good inhibition observed in all extracts tested. The activity was compared with standard antibiotics chloramphenicol, ethryomycin and methicillin. All the three were found to be multi-drug resistant isolates. The zone of inhibition was measured in mm and tabulated in Table 2. A higher inhibition was in case of *Pseudomonas* at higher concentration of 100µg tested. In case of isopropanol extract, there was higher inhibition observed against *E.coli* compared to *Pseudomonas.*

Antimicrobial activity of other parts of *E. rheedii* were evaluated previously. Antimicrobial activity of seed extract was studied and no significant antimicrobial and thrombolytic activitieswereobserved **[12]**. However, this is the pioneer work on leaf extract showing antimicrobial activity even at low concentration. This was proved to be effective against multidrug resistant pathogens which are a significant finding. Antimicrobial study of *Entada scandens (E. scandens)* also revealed that there was no significant inhibition was found *against Escherichia coli, Pseudomonas aureus, Plesiomonasshigelloides, Salmonella typhi, S. paratyphi, Shigelladysenteriae, S. flexneri, S. boydii, S. sonnei, Proteus vulgaris, Enterococcus faecalis, Staphylococcus saprophyticus, S. aureus, S. epidermidis and Streptococcus pyogenes**[11].***

Conclusions

This study revealed that the various solvent extract of leaves of *E. rheedii* were suitable as an antimicrobial agent against *E.coli, Pseudomonas, Bacillus* sp. and *S. typhi*. In case of Pseudomonas, there was significant inhibition observed at highest concentrations tested. The phytochemical analysis revealed that the extract possessesflavonoids, tannins, terpenoids etc. which may be attributed to the antimicrobial effect of the extract. This is a first report on antimicrobial activity of leaf extract of *E. rheediieffective* against multidrug resistant bacterial isolates.

Table 2. Antimicrobial efficacy of leaf extract of E. rheedii against selected pathogenic bacteria

Solvents	Microorganisms	Extract Concentration			
		25µl	50 µl	75 µl	100 µl
Methanol	*Bacillus*	14 ± 0.14	16 ± 0.28	20 ± 0.11	23 ± 0.16
	E.coli	17 ± 0.27	19 ± 0.13	22 ± 0.18	24 ± 0.11
	Pseudomonas	16 ± 0.15	21 ± 0.18	25 ± 0.24	32 ± 0.13
	S.typhi	17 ± 0.32	19 ± 0.24	21 ± 0.33	23 ± 0.14
Chloroform	*Bacillus*	10 ± 0.22	14 ± 0.26	20 ± 0.12	22 ± 0.23
	E.coli	11 ± 0.34	14 ± 0.22	19 ± 0.32	21 ± 0.27
	Pseudomonas	9 ± 0.21	15 ± 0.08	25 ± 0.22	34 ± 0.12
	S.typhi	12 ± 0.19	14 ± 0.14	17 ± 0.11	24 ± 0.18
Ethanol	*Bacillus*	10 ± 0.12	13 ± 0.18	17 ± 0.05	21 ± 0.14
	E.coli	9 ± 0.17	11 ± 0.34	16 ± 0.10	20 ± 0.18
	Pseudomonas	12 ± 0.36	16 ± 0.13	19 ± 0.13	22 ± 0.25
	S.typhi	8 ± 0.17	12 ± 0.17	15 ± 0.20	18 ± 0.19
Isopropanol	*Bacillus*	10 ± 0.12	14 ± 0.11	16 ± 0.14	18 ± 0.23
	E.coli	12 ± 0.27	16 ± 0.18	18 ± 0.17	26 ± 0.14
	Pseudomonas	7 ± 0.19	13 ± 0.21	16 ± 0.23	20 ± 0.15
	S.typhi	11 ± 0.13	14 ± 0.23	15 ± 0.19	20 ± 0.28

[Values represent Mean ±S.D of triplicate experiments]

Acknowledgements

Authors extend acknowledgement to the AA Government Arts College, Namakkal, India for necessary infrastructure facilities.

References

1. MO Mona, MS Fathy, SE Kadriya andFY Miriam. Botanicalstudy, DNA fingerprinting, nutritional values and certain proximates of Entada rheedii Spreng. Int. J. Pharm. Pharm. Sci. (2013;5(3),311–329.
2. ZU Ahmed. Encyclopedia of flora and fauna of Bangladesh, vol 9. Asiatic Society of Bangladesh, Dhaka, 2009; 164–165.
3. LK Nzowa, L Barboni, RB Teponno, M Ricciutelli, G Lupidi, L Quassinti, M Bramucci andLA Tapondjou.Rheediinosides A and B antiproliferative and antioxidant triterpene saponins from Entada rheedii. Phytochem.2010; 71, 254–261.
4. BP Gaire and L Subedi. Medicinal plant diversity and their pharmacological aspects of Nepal Himalayas. Pharmacognosy J. 2011; 3(25), 6-17.
5. M Brink and EG Achigan-Dako. Fibres PROTA foundation. Wageningen, Netherlands, 2012; 152–154.
6. M Shafaat-Al-Mehedi, CM Hasan and MR Haque. Isolation of flavonoids from the bark of Entada rheediiSpreng. Oriental Pharmacy and Experimental Medicine. 2015; 15(4), 347-351.
7. LK Nzowa, RB Teponno, LA Tapondjou, L Verotta, Z Liao, D Graham, MC Zinkc and L Barboni. Two new tryptophan derivatives from the seed kernels of Entada rheedei: effects on cell viability and HIV infectivity. Fitoterapia. 2013; 87,37–42
8. S Sugimoto, K Matsunami and H Otsuka. Medicinal plants of Thailand. I: structures of rheedeiosides A-D and cis-entadamide A-β-D-glucopyranoside from the seed kernels of Entada rheedei. Chem. Pharm. Bull. 2011; 59,466-471
9. N Srividya N and M Chandra. Evaluation of bioactive compounds and antioxidant activity of some wild fruits by in-vitro assays.Int. J. Pharma. Sci. Res. 2015; 6(1), 233.
10. JimThomas, M Suresh Kumar, N Vinod Kumar, EG Wesely and M Rajasekara Pandian. Antimicrobial activity and phytochemical evaluation of aqueous extract of Artocarpushirsutus Lam. Bark. Global J. Res. Analysis. 2016; 5(6),
11. KDey, M Rahman, A Hira, A Ahmed, MS Howlader, A Khatun and MASiraj. Phytochemical screening and pharmacological activities of Entada scandens seeds. Int. J. Appld. Res. Nat. Prodt. 2013;6(1), 20-26.
12. Md. Abu Sufian, Md. Shafaat -Al-Mehedi, Mohammad AbdurRashid and Mohammad RashedulHaquebiological investigation of Entada rheediispreng. And isolation of entadamide a from its seed. Int. Res. J. Pharm. 2015;6 (7), 411-414.