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COMPARATIVE STUDY OF ANTIBACTERIAL POTENTIAL OF *IN VIVO* AND *IN VITRO* ROOT EXTRACTS OF *MUCUNA GIGANTEA* (WILLD.) DC.-AN ENDANGERED MEDICINAL PLANT OF ODISHA

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ABSTRACT ■ The present study compares antibacterial potential of *in vivo* (natural) and *in vitro* (tissue culture regenerated) root extracts of *Mucuna gigantea*. The antibacterial activity was performed by agar-well diffusion method against five different human pathogenic bacterial strains, viz. *Streptococcus pyogenes* (MTCC 1926), *Streptococcus mutans* (MTCC 497), *Salmonella typhii* (MTCC 1252), *Sigella flexneri* (MTCC 1457) and *Vibrio cholera* (MTCC 0139). The antibacterial activity of aqueous and methanolic extracts of both *in vivo* and *in vitro* root specimens was determined by measuring the diameter of inhibition zone. Methanol-root extracts of both *in vivo* and *in vitro M. gigantea* showed greater zone of inhibition than aqueous extracts. Among the five bacterial strains used, root extract (*in vivo* and *in vitro*) showed maximum zone of inhibition against *Sigella flexneri*. Of the two types of root extracts, *in vitro* root showed higher zone of inhibition i.e. 2.3cm than in *in vivo* root which showed 2.0cm at 500 mg/ml of concentration for *S. flexneri*. *In vitro* root exhibited better antibacterial activity than *in vivo*- root. As the findings confirmed that both aqueous and methanol *in vitro* root extracts are capable of showing higher zone of inhibition than the *in vivo* extracts, credence must be given to the use of *in vitro* root of *M. gigantea* as continuous and sustainable resources for pharmaceutical industries so that the endangered medicinal mangrove plant of Odisha can remain undisturbed.

Key words: Endangered medicinal mangrove plant, Human pathogenic bacterial strains, *In vitro* root extract, Zone of inhibition, *M. gigantea*

INTRODUCTION

To date, the herbal medicine is still the pillar of about 75 to 80% of the world population, mainly in the developing countries, for primary health care because of better compatibility with the human body and lesser side effects (Deokar *et al*., 2016). The medicinal values of the plants lie mostly in their secondary metabolites, which produce definite physiological actions on the human body and are potential sources of useful drug as well as other useful bioactive products (Ujowundu *et al*., 2010; Ramawat *et al*., 2009). The medicinal plants contain innumerable biologically active chemicals or secondary metabolites with antibacterial activity and medicinal properties (Viji *et al.,* 2010). In developing countries where medicines are

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quite expensive, investigation on antimicrobial activities from ethno medical plants may still be needed (Cos *et al*., 2006). *Mucuna gigantea* L. (DC) is indigenous to tropical regions especially Africa, India and West Indies, America, Mexico and Eastern Nigeria (Marimuthu, 2015). In India, It grows wildly in the coastal regions of Tamil Nadu, Kerala and Andaman regions (Janardhanan *et al*., 2003). In Odisha it is very much found in Bhitarkanika. A decoction of the root is taken to treat gonorrhoea and schistosomiasis (Rajaram *et al.*, 1991; Brink, 2006). The powdered bark is mixed with dry ginger and used in rheumatic complaints by rubbing it over the affected parts (Brink, 2006). The powdered seed is used as a purgative (Brink, 2006). The proximate composition (crude proteins, crude lipids, ash, nitrogen free extractives :: 30.62, 9.03, 5.99, 42.79 % respectively) and nutritional potential (100 g dry seed is 374.91 kcal) of *Mucuna gigantea* seeds were investigated by Rajaram and Janardhanan(1991). The essential amino acid like leucine and isoleucine are present relatively in large quantities (Janardhanan *et al*., 2003) whereas, seed contains 1.7-2.0% L-DOPA (Levodopa; L-3,4-dihydroxyphenylalanine), a non protein amino acid which stimulates the formation of the neurotransmitter dopamine in the brain (Brink, 2006). The L-DOPA concentration, in *Mucuna gigantea* has been found to be relatively very high compared to other species of *Mucuna*, which leads to anti-parkinson's effect (Soares *et al*., 2014). The boiled seeds with reduced L-DOPA are known to be consumed by Oceanic group of tribal's like Onges, Great Andamanese, Sompens etc. To the best of our knowledge there was no earlier report on antimicrobial activities of *M. gigantea* as it is known for other species of *Mucuna*. As such the present study has been kept concerned with the comparative study of antibacterial activity of natural and tissue cultured plant specimens on certain pathogenic bacterial strains.

MATERIALS AND METHODS

Collection and preparation of plant extracts

Two different types of root materials of *M. gigantea* i.e. *in vivo* (root from 10 month old plant) and *in vitro* (roots from tissue culture derived 05 month old plant) were collected from the experimental garden of the Department of Botany, Ravenshaw University, Cuttack and their extracts were used for the antibacterial studies.

10 gm of powdered roots of both *in vivo* and *in vitro* were extracted with two different solvents (250 ml each of aqueous and methanol) by Soxhlet apparatus (Borosil, India). All the extracts were evaporated at room temperature and stored in sterile glass vials at 4°C for antibacterial assay (Fig. 1). 1000 mg of each crude plant extract (aqueous and methanol) was dissolved with 1ml of 100% DMSO for the preparation of stock solution. Further different concentrations like 500 mg/ml, 250 mg/ml, 125mg/ml, 62.5 mg/ml and 31.25 mg/ml were prepared from the stock solution by diluting with DMSO.

Fig. 1. Roots of *M. gigantea* and their extracts

Test bacterial strains

For the present antibacterial study five different human pathogenic bacterial strains were used which were procured from IMTECH, Chandigarh. Of these specimens two are gram positive bacteria [*Streptococcus pyogenes* (MTCC 1926) & *Streptococcus mutans* (MTCC 497)] and three gram negative bacteria [*Salmonella typhii* (MTCC 1252), *Sigella fixinaria* (MTCC 1457) and *Vibrio cholera* (MTCC 0139)]. All strains were maintained on nutrient agar slants. Each bacterial culture was further maintained by sub-culturing regularly on the same medium and stored at 4°C for experiment. Agar well diffusion method was used to determine the antibacterial activity of both the types (*in vivo* and *in vitro*) root extracts.

Preparation of bacterial inocula

Nutrient broth (Hi media, Mumbai, India) was used to maintain broth culture. Selected colonies of aforesaid bacteria were picked off from a fresh isolation plate and inoculated in corresponding tubes containing about 5 ml of the broth. After inoculation these tubes were incubated for 6±1hours at 35±2ºC until there was visible growth. Mc Farland 0.5 standard was used to get 10^5 colony-forming units (CFU/ml) by adjusting the turbidity.

Anti-bacterial activity assay using agar well diffusion method

Agar well diffusion method is widely used to determine the antimicrobial activity of plant extracts (Balouiri *et al*., 2016). As such, 20 ml of Nutrient agar medium was poured into sterile petri dishes and allowed for solidification. Nutrient agar plates were swabbed using sterile cotton swabs with broth culture of each individual bacterial strain. Then, wells of 0.7 cm diameter were made aseptically in each plate. About 100 μl of each root crude solvent extract was loaded into the wells. The agar plates were left undisturbed, at room temperature for a few hours, allowing the extracts to diffuse into the agar medium (Sen and Batra, 2012). The plates were incubated at 35±2 °C, overnight and the zones of inhibition (cm) were measured (Perez *et al*., 1990). The antibiotic sensitivity test was done by using standard Kanamycin (Himedia, India) and DMSO was used as negative control.

RESULTS AND DISCUSSIONS

Total five human pathogenic bacterial strains were taken into consideration causing different diseases like tooth decay, infection and several skin infections and pharyngitis by *S. mutans* and *S. pyogenes*. Other three *S. typhii, S. flexneri* and *V. cholera* are capable of causing typhoid, diarrhoea, cholera respectively. The complete result of antibacterial assay by agar well diffusion method against five human pathogenic bacteria was presented in Table 1.

Aqueous crude root extracts (*in vivo* and *in vitro*) did not show any antibacterial activity against any strain at 31.25 mg/ml whereas, methanol crude root extracts (*in vivo* and *in vitro*) were found effective against *Shigella flexneri*. Overall, methanolic root extract of both the types (*in vivo* and *in vitro*) showed better result than the root aqueous extract of both the types at all most all concentrations. So all the two methanolic *in vivo* and *in vitro* root crude extracts could exhibit highest inhibition zone against *Shigella flexneri.* At 500mg/ml *in vivo* and *in vitro* root showed up to 2.0cm in and 2.3cm respectively against *Shigella flexneri* (Fig.2: I, II, III & IV). Due to lack of literature about *Mucuna gigantea*, another species of genus *Mucuna* i.e. *M. Pruriens* was considered. Bala *et al*.(2011) reported lowest inhibitory potential of ethanolic extract of aerial parts of *M. puriens* against *Shigella flexneri*. The strong antibacterial efficiency of our methanolic extracts also corroborated with the findings

Table 1. Antibacterial activity of *in vivo* and *in vitro* root extracts (mg/ml) of *M. gigantea*

Different solvents	Bacterial Strains	Zone of inhibition (cm) of *in vivo* root				
		500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml
Aqueous	SF	1.4±0.05	1.1±0	? 0.9±0.1	NI	NI
	SM	1.3±0	1.2±0.05	? 0.9±0.1	NI	NI
	SP	1.5±0	1.4±0.05	1.1±0	NI	NI
	ST	1.6±0.05	1.3±0	1.2±0.05	1.0±0	NI
	VC	1.6±0.05	1.3±0	1.2±0.05	1.0±0	NI
Methanol	SF	2.0±0.05	1.7±0.05	1.6±0.05	1.5±0	? 0.9±0.1
	SM	1.7±0.05	1.6±0.05	1.4±0.05	1.3±0	? 0.9±0.1
	SP	1.5±0	1.4±0.05	1.3±0	1.0±0	NI
	ST	1.8±0.05	1.6±0.05	1.1±0	? 0.9±0.1	NI
	VC	1.7±0.05	1.6±0.05	1.0±0	? 0.9±0.1	NI

Zone of inhibition (cm) of *in vitro* root					Kanamycin
500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	10 μg/ml
1.5±0	1.3±0	1.2±0.05	1.1±0	NI	2.5±0.1
1.5±0	1.5±0	1.3±0	? 0.9±0.1	NI	2.1±0
1.5±0	1.6±0.05	1.5±0	1.3±0	NI	2.6±0.1
1.8±0.05	1.3±0	1.2±0.05	1.2±0.05	NI	2.0±0.05
1.8±0.05	1.5±0	2.5±0.1	1.3±0	NI	2.0±0.05
2.3±0.1	1.9±0.05	2.1±0	1.3±0	? 0.9±0.1	2.5±0.1
2.0±0.05	1.9±0.05	2.6±0.1	1.5±0	? 0.9±0.1	2.1±0
1.5±0	2.0±0.05	2.0±0.05	1.3±0	NI	2.6±0.1
2.0±0.05	1.8±0.05	1.7±0.05	1.5±0	NI	2.0±0.05
2.0±0.05	1.9±0.05	1.7±0.05	1.3±0	NI	2.0±0.05

NI- No inhibition, SF-*Shigella flexneri,* ST-*Salmonella typhii*, SM-*Streptococcus mutans*, VC- *Vibrio cholerae*, SP-*Streptococcus pyogenes*

Inhibition zone diameters are expressed as Mean (n=3) ± SE

of Salau and Odeleye (2007) who reported about the efficiency of methanolic extract of *M. Pruriens* leaves. They depicted about its strong antibacterial activity against *S. aureus*, *E. coli*, *B. subtilis*, *Proteus mirabilis*, *P. aeruginosa*. When Apparao Rayavarapu and Kaladhar (2011) examined the antibacterial efficacy of methanolic, hexane and chloroform extracts of *M. pruriens* leaves, they also found that methanolic leaf extract showed highest antimicrobial activity than other two extracts against all tested bacteria such as *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas marginalis*, *Pseudomonas aeruginosa*, *Xanthomonas campestris*. Similarly Pujari and Gandhi (2013) have also reported about stronger antibacterial activities of the methanol extract of leaves of *M. pruriens* against *Staphylococcus aureus, Salmonella typhii*, *Shigella dysenteriae*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* compared to ethanol- and acetone- extracts at concentration of 0.05 g/ml At 500mg/ml, Methanol root extracts of both the types (*in vivo* and *in vitro*) showed lowest zone of inhibition against *S. pyogenes*. Aqueous root showed lowest against *S. mutans* but interestingly *in vitro* root extract showed (1.5cm) better results than *in vivo* (1.3cm).

In vitro root extracts revealed better antibacterial activity than *in vivo* root extracts. Methanolic *in vitro* root extract revealed 2.3cm

zone of inhibition which is higher than *in vivo* root extract which was 2.0cm at 500 mg/ml against *Shigella flexneri.* Aqueous extract of *in vitro* root showed best inhibition against *S. typhii* and *V. cholerae* with zone of inhibition diameter 1.8cm which is greater than the diameter 1.6cm exhibited by aqueous *in vivo* root extract showed at 500mg/ml of drug concentration. But when Murugan and Mohan (2012) evaluated the antibacterial efficacy of hexane, petroleum ether, benzene, methanol and water extracts of root and seed of *Mucuna atropurpurea* against *Salmonella typhii* along with other five bacterial strains they found no inhibition zone around aqueous extract of *M. atropurpurea* root against the *Salmonella typhii* which is contrary to our result.

From a few available literature on *M. gigantea*, it could be known that the decoction of the root is used to treat gonorrhoea caused by a bacterium *Neisseria gonorrhoeae* (Brink, 2006). It supports our result that the same root extract shows effective antibacterial activity against *Neisseria gonorrhoeae*. The antibacterial activity showed by different crude extracts in our experiment is likely to be due to the presence of different types of secondary metabolites. Perhaps the better result obtained with the *in vitro* root extract than with *in vivo* root extract may be due to the presence of higher quantities of secondary metabolites in roots of tissue culture derived plants.

Though both *in vivo* and *in vitro* plants are collected from natural condition but, the possible reasons for the enhanced levels of active compounds in tissue culture raised plants could be, the tissue culture plants are grown under controlled growth and environmental conditions receiving an optimum supply of nutrients, plant growth regulators and other favorable growth condition (Chen *et al.*, 2014). Like the present work, Moharana *et al*.(2014) have compared the antibacterial activities of both *in vivo* and *in vitro* leaves of *Lawsonia inermis* against four pathogenic bacteria by broth dilution method. They reported about stronger antibacterial activity of *in vitro* leaves than of *in vivo* leaves.

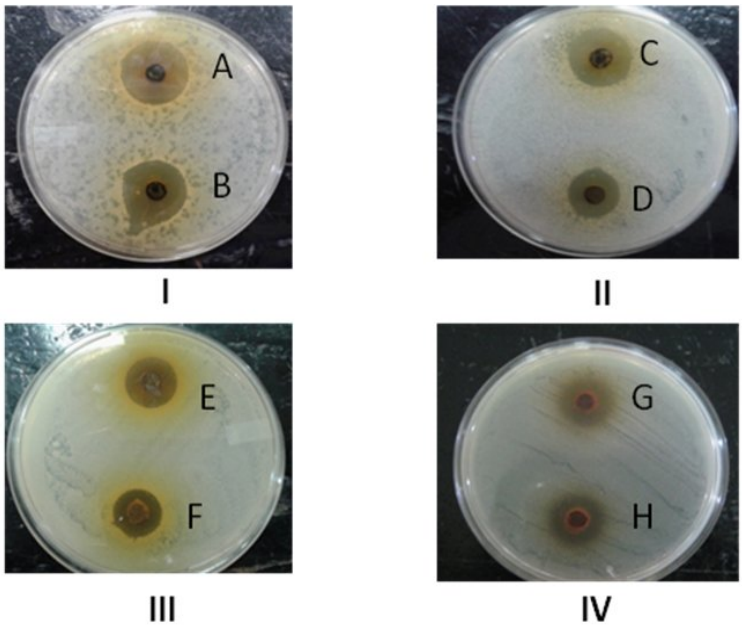

Fig. 2
I- Inhibition zone (cm) of methanol *in vitro* root extract on *S. flexneri* (A & B are replica)
II- Inhibition zone (cm) of methanol *in vivo* root extract on *S. flexneri* (C & D are replica)
III- Inhibition zone (cm) of aqueous *in vitro* root extract on *S. flexneri* (E & F are replica)
IV- Inhibition zone (cm) of aqueous *in vivo* root extract on *S. flexneri* (G & H are replica)

Though *M. gigantea* has several uses in food as well as in medicine, there is always the chance of over exploitation by local people and pharmaceutical companies. Due to the slow propagation and low seed viability, it is not profusely available to fulfill their increasing commercial demands. Moreover for its endangered status the species requires attention for conservation. Tissue culture needs to be adopted since it offers *in vitro* regeneration of plants in large scale in less time with a small piece of tissue, cell or organ without disturbing the plants in natural habitats. Like other species of *Mucuna* , the species also needs to be further explored for use in food-, pharmaceutical- and other industries.

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