Publication Ref No.: IJPRD/2010/PUB/ARTI/VOV-2/ISSUE-10/DEC/010 ISSN 0974 – 9446

International Journal of Pharma. Research & Development - Online (IJPRD)
Platform for Pharmaceutical Researches and Developments
International Standard Serial Number : 0974 - 9446
www.ijprd.com

IN VIVO AND *IN VITRO* ANTI-BACTERIAL EFFICACY OF *ALTERNANTHERA SESSILIS* (LINN.)

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ABSTRACT

The present study was under taken to compare the antibacterial potential of leaves, inter-nodal segments, leaves and inter-nodal segments derived calli of *Alternanthera sessilis* (Linn.) R.Br. ex DC. Maximum percentage of callus formation (inter-nodal segments 88.9 ± 0.83; leaves segments 92.4 ± 0.61) was obtained on Murashige and Skoog's basal medium supplemented with 3% sucrose and 0.5 mg/l of 2, 4 - Dichlorophenoxy acetic acid. Dried whole plants as also the *in vitro* derived callus were powdered using the electric homogenizer exhaustively extracted by cool extraction with ethanol, Ethyl acetate, petroleum ether and water for 72 h. Antimicrobial activity was determined against four bacterial pathogens by the agar disc diffusion assay. The antibacterial effect of the leaves, inter-nodal, leaves and inter-nodal segments derived calli of *A. sessilis* was evaluated against *Proteus vulgaris, Streptococcus pyogenes, Bacillus subtilis* and *Salmonella typhii.* The ethanolic extracts of leaves and leaves derived calli were more effective against the selected bacteria than other and solvents.

Key Words: *In vitro*, *In vivo*, Bio- efficacy, Anti-bacterial, Callus .

INTRODUCTION

India throughout its long history has accumulated a prosperous practical acquaintance of the use of medicinal plants for the treatment of various diseases. Chemical studies of Indian medicinal plants make available a priceless material foundation for the discovery and development of new drugs of natural origin. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to cure many infectious diseases (1-3). Botanists, Phytochemist and Pharmacologists are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against cancer, as well as viral and microbial infections (4). There are more than 35,000 plant species being used in various human cultures around the world for medicinal purpose (5). Although thousands of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated (6). According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. In recent years, pharmaceutical companies have spent considerable time and money in developing therapeutics based upon natural products extracted from plants (7, 8). The rising incidence of multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources (9, 10). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency (11-20). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils (21), as well as in tannin (22).

Plants from the genus *Alternanthera* are thought to possess antimicrobial and antiviral properties. In Brazilian folk medicine, the aqueous extract of *A. tenella* Colla is used for its anti-inflammatory activity. Guerra *et al*., (23) reported the immunomodulatory property and antitumor activity of *A. tenella* extract. *A. spinosus* is used for anti-malarial, anti-ulcer and anti-inflammatory (24, 25). Sunil *et al*., (26) reported the wound healing property of *Alternanthera sessilis.* Gayathri *et al*., (27) revealed degenerative and necrotic changes in the liver and kidney in Swiss mice, caused by oral administration of water extract of *A. sessilis* in high doses through histopathological test. In recently, a lot of studies related to antimicrobial activities of plant extracts (28-32) and callus extracts (33-39). *Alternanthera sessilis* (L.) DC. (Sessile joy weed; *Amaranthaceae* is a popular leafy vegetable in Sri Lanka and also used as traditional medicine in China, Taiwan, India and Sri Lanka. With this background an attempt was taken to compare the antibacterial potential of leaves, inter-nodal segments, leaves and inter-nodal segments derived calli of *Alternanthera sessilis* (Linn.) R.Br. ex DC.

MATERIALS AND METHODS

Callus induction

Inter-nodal and leaves segments of *Alternanthera sessilis* (L.) DC. were collected from the young top shoot cuttings of mature plants. The explants were washed thoroughly under running tap water for 5 min. and then washed with a commercial detergent tween-20 for 60 sec. followed by thorough washing with sterile distilled water. Surface sterilization was done with mercuric chloride solution (0.5 % w/v) for 60 sec. then washed thrice with sterile distilled water, the explants were cut into 0.7 cms in length and cultured on Murashige and Skoog (40) solid medium supplemented with 3% sucrose, gelled with 0.7% agar and different concentration of 2, 4 - D either alone or in combination. The pH of medium was adjusted to 5.8 before autoclaving at a pressure of 1.06 Kg/cm^2 (121^{o}C for 15 min.). The cultures were incubated at 25^o ± 2^{o}C with 12/8 h photoperiod under white fluorescent tubes (1500 lux). Each and every experiment was performed with ten replicates and repeated thrice. The callus cultures were maintained for a period of over 10 months by periodic sub-culturing with 2 to 4 weeks intervals on to fresh multiplication medium. Growth and phytochemical assays were performed at the 21st day. Consequently, the callus were harvested at the transfer age of 3 to 4 weeks, kept at above 90℃ for 3 to 5 minutes at hot air oven to inactivate the enzyme activity followed by continuous drying at 50 to 60℃, till a constant weight was obtained and these callus were further exploited for extraction and bacterial efficacy analysis. Dried whole plants as also the *in vitro* derived callus were powdered using the electric homogenizer exhaustively extracted by cool extraction with ethanol, Ethyl acetate, petroleum ether and water for 72 h (41). The solvent-containing extracts were decanted and filtered. All the extracts and fractions were stored at 4℃ for antibacterial activity.

Antibacterial Activity

Stock cultures were made fresh every seven days on agar slants during this scheme of work. Pure bacterial cultures viz., *Bacillus subtilis, Proteus vulgaris*, *Streptococcus pyogenes* and *Salmonella typhi* were maintained on nutrient broth at 37℃ for 24 hrs. Bacterial strains were cultivated at 37℃ and maintained on nutrient agar (Difco, USA) slant at 4℃. Antimicrobial activity was determined against four bacterial pathogens by the agar disc diffusion assay (42). The crude and fractionated extracts were dissolved in Dimethyl Sulfoxide (DMSO) with the exception of the water fraction and then antimicrobial effects of crude fractionated extracts were tested using four different concentrations viz., 100, 250, 500 and 1000 µg. Petri dishes (measuring 90 mm each side) containing 20 ml of Mueller Hinton agar (Himedia, Mumbai). At the same time, 6 mm diameter sterile Whatman Antibiotic disc were placed on the surface of the inoculated agar plates, and then appropriate concentration of the extracts in DMSO and water were applied onto the discs, 100, 250, 500 and 1000 µg final concentrations were obtained for each discs. The plates were incubated at 37℃ for 16-18 h. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs. Standard discs of the antibiotic gentamycin (10 mg) and ampicilin (10 mg) served as the positive antibacterial controls. Negative controls were done using paper discs loaded with 20 ml of DMSO and water. The experiments were repeated in triplicate and the inhibition zone and antibacterial activity s were documented.

RESULTS AND DISCUSSION

Callus induction was observed on the inter-nodal and leaves segments on MS medium supplemented with 2, 4-D. Based on the concentration of plant growth hormone the callus formation frequency was varied (Table - 1). Maximum percentage of callus formation (inter-nodal segments 88.9 ± 0.83; leaves segments 92.4 ± 0.61) was obtained on Murashige and Skoog's basal medium supplemented with 3% sucrose and 0.5 mg/l of 2, 4 - Dichlorophenoxy acetic acid. Different types of calli were obtained of which, the friable, semi friable and creamy white coloured showed high proliferation rate. In high concentration of auxins the callus was hard and dark yellowish brown in colour. The semi friable callus was showed highest rate of shoot proliferation. Friable calli were showed highest percentage of cell division and cell multiplication. Leaves derived calli more friable than the inter-nodal segments derived calli and they showed high frequency of proliferation rate. Influence of 2, 4-D on callus induction was observed by number of tissue culturist (38, 43-44). In the present study we observed maximum percentage of callus on 2, 4-D supplemented medium. Our result was directly consonance with the

Manickam *et al*. [44] observation on *Withania somnifera*.

Several workers have reported that many plants possess antimicrobial properties including the parts which include; flower, bark, stem, leaf, etc. It has been shown that when solvents like ethanol, petroleum ether, chloroform, isopropanol, hexane and methanol are used to extract plants, most of them are able to exhibit inhibitory effect on both gram positive and gram negative bacteria (45). In the present study also we observed the anti-bacterial activity of leaves, inter-nodal segments, leaves and inter-nodal segments derived calli of *A. sessilis.* The results of antibacterial screening tests of leaves and inter-nodal segments, leaves and inter-nodal segment derived calli extracts of *A. sessilis* in different solvents viz., methanol, petroleum ether, Ethyl acetate and water against pathogenic bacteria using disc diffusion techniques are depicted in Table - 2 and 3. The ethanol extracts of leaves and leaves derived calli showed the maximum bio-efficacy compared with other solvents (Table - 3). Phytochemical constituents such as tannins, saponins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and other herbivores (46-49). This can partially explain the demonstration of antimicrobial activity by the stem and leaf extracts of *Polygonum aviculare, Anogeissus leoicarpus, Amaranthus hybridus, Passiflora mollussima, Phyllanthus amarus, Passiflora edulis, Rauvolfia tetraphylla* and *Physalis minima* (29, 31, 34, 36, 38 and 39). The demonstration of antimicrobial activity against both Gram-positive and Gram-negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds (32).

Observation of the present study was supported by the previous observation on *Phyllanthus amarus, Rauvolfia tetraphylla*, *Physalis minima Passiflora mollussima*, *Passiflora edulis* leaf and callus extracts (34, 36, 38 and 39). Earlier observations on *Baliospermum axillare Rauvolfia tetraphylla*, *Physalis minima* and *Mimosa hamata* leaf and callus extracts showed considerable antibacterial and antimicrobial activity (35-37). The present observation augments the previous bio-efficacy studies on cell cultures. The present study observation is strengthening the bio-efficacy studies on cell cultures. Antibacterial effects of these plants on Proteus sp. showed that the plants can be used in the treatment of urinary tract infection associated with *Proteus* sp. (50). Through the bacterial efficacy studies, it is confirmed that the *in vitro* raised calli tissue was more effective compared to *in vivo* tissue. The ethanolic extracts of leaves and leaves derived calli were more effective against the selected bacteria than the inter-nodal segments. Potentially viable and reproducible callus cultures to biosynthesize similar compounds are established. Further work is required to find out the active principle from the plant extracts and to carry out pharmaceutical studies.

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TABLES:

Table 1: Effect of 2, 4-D on Callus production from the Leaves and Inter-nodal segments of *Alternanthera sessilis* L

MS medium + 2, 4 – D in mg/l	Mean percentage of callus induction ± S.E.		Type of Callus	
	Leaves	Inter-nodal	Leaves	Inter-nodal
0.0	00.0 ± 0.0	00.0 ± 0.0	NIL	NIL
0.1	46.4 ± 0.64	37.6 ± 0.43	Friable	Semi-friable
0.3	68.4 ± 0.94	59.7 ± 0.48	Friable	Semi-friable
0.5	92.4 ± 0.61	88.9 ± 0.83	Friable	Semi-friable
1.0	83.4 ± 0.86	80.7 ± 0.67	Semi-friable	Semi-friable
1.5	71.8 ± 0.64	70.6 ± 0.56	Semi-friable	Semi-friable
2.5	61.1 ± 0.58	63.4 ± 0.47	Semi-friable	Semi-friable
3.0	52.1 ± 0.67	48.7 ± 0.64	Semi-friable	Semi-friable

Table 2: *In vivo* and *in vitro* antibacterial efficacy leaves extracts of *A. sessilis*

Solvents	Conc.	*Leaves Extracts*				*Leaves derived Calli extracts*			
		S. t	*S.p*	*P.v.*	*B.s*	*S. t*	*S.p*	*P.v.*	*B.s*
Pet. Ether	100	10	00	00	12	12	00	00	12
	250	17	00	00	14	18	00	00	14
	500	18	00	00	17	20	00	00	18
	1000	20	00	00	23	23	00	00	26
Ethyl acetate	100	00	10	00	12	00	10	00	12
	250	10	13	14	16	11	13	14	16
	500	14	16	16	19	15	16	17	21
	1000	17	19	18	25	19	19	19	27
Ethanol	100	10	10	11	10	10	10	11	10
	250	11	15	15	15	13	15	15	15
	500	18	17	18	18	20	17	18	18
	1000	**27**	**22**	**20**	**25**	**28**	**22**	**20**	**25**
Water	100	00	10	00	13	00	10	00	13
	250	00	13	00	16	00	13	00	16
	500	13	17	14	19	13	17	14	19
	1000	14	21	16	22	14	21	16	22

S. t – Salmonella typhi ; S.p – Streptococcus pyogenes; P.v.- Proteus vulgaris; B.s – Bacillus subtilis

Table 3: Antibacterial efficacy of inter-modal and inter-nodal derived calli of *A. sessilis*

Solvents	**Conc.**	**Inter-nodal Extracts**				**Inter-nodal derived Calli extracts**			
		S. t	*S.p*	*P.v.*	*B.s*	*S. t*	*S.p*	*P.v.*	*B.s*
Pet. Ether	100	14	00	00	12	14	00	00	12
	250	18	00	00	15	18	00	00	15
	500	22	00	00	17	22	00	00	17
	1000	29	00	00	23	29	00	00	23
Ethyl acetate	100	13	00	00	13	13	00	00	13
	250	20	00	00	17	20	00	00	17
	500	30	00	00	20	30	00	00	20
	1000	34	00	00	22	34	00	00	22
Ethanol	100	10	10	00	12	10	10	00	12
	250	16	15	00	15	16	15	00	15
	500	28	17	00	19	28	17	00	19
	1000	**35**	**19**	**00**	**26**	**35**	**19**	**00**	**26**
Water	100	00	00	00	12	00	00	00	12
	250	10	00	00	14	10	00	07	14
	500	14	00	12	18	14	00	13	18
	1000	15	00	17	20	15	00	18	21

S. t – Salmonella typhi ; S.p – Streptococcus pyogenes; P.v.- Proteus vulgaris; B.s – Bacillus subtilis