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Antibacterial studies on *in vitro* derived calli of *Ocimum basilicum* L.

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ABSTRACT

The present study was aimed to screen the antibacterial potential of calli derived from inter-nodal and leaf segments of Ocimum basilicum. Highest percentage of callus formation (inter-nodal segments 75.2 ± 0.90; leaves segments 78.7 ± 0.51) was obtained on Murashige and Skoog's basal medium supplemented with 3% sucrose and 1.0 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. Five different solvent extracts of in vitro derived calli were examined for the antibacterial activity against the isolated human pathogens and the results are given in the table - 2. The maximum zone of inhibition 12 mm for Salmonella typhi, 13 mm for Klebsiella pneumoniae, 17 mm for Proteus vulgaris, 18 mm for Pseudomonas aeruginosa and B. subtilis and 10 mm for Staphylococcus aureus were observed. The ethanol extracts of leaf derived calli of O. basilicum illustrated the high degree of activity (5/6). The methods developed in this work make possible for the low volume and high potential production of active principles under in vitro condition in short duration with less amount of explants utilization.

Keywords: *in vitro*, Calli, Bio-efficacy, Antibacterial.

INTRODUCTION

Many higher plants are major sources of natural products used as pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives, and pesticides [1]. Studies on plant secondary metabolites have been increasing over the last 50 years. Moreover, different national and international pharmaceutical companies are utilizing such plant based formulations in treatment of various diseases and disorders worldwide [2-4]. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases [5]. Recently, much attention has been directed toward extracts and biologically active compounds isolated from popular plant species [6-8]. Plants may be considered as a famous chemical factory for biosynthesis of a huge array of

secondary metabolites [9]. The so-called secondary metabolites have contributed more than 7000 different compounds in use today as cardiac drugs, anticancer agents, hormones, antibiotics, laxatives, diuretics, analgesics, anaesthetics, drugs for ulcer treatments and antiparasitic compounds. In USA, 74% of drugs are based on plants [10]. The accumulation of phytochemicals in the plant cell cultures has been studied for more than thirty years, and the generated knowledge has helped in the realization of using cell cultures for production of desired phytochemicals [11]. The global demand of plant origin bioactive compounds is very high, but not possible to fulfill by field grown plants.

An attractive and very promising alternative system for commercial exploitation is plant cell cultures thereby producing high yield compared to field grown plants [12]. Currently the various applications of genetic engineering works are implemented in medicinal plants to increase the secondary metabolites production [13]. The genus *Ocimum* belongs to the family Lamiaceae and is comprised of more than 50 species of herbs and shrubs distributed in tropical and subtropical regions of Asia, Africa and the Americas. Ocimum is used as a stomachic, antihelmintic, antipyretic, diaphoretic, expectorant, carminative, stimulant and pectoral. It is also used in the purulent discharge of the ear, bronchitis, hiccough and diseases of the heart and brain [14]. *Ocimum basilicum* L. (Lamiaceae), commonly known as sweet basil is an evergreen multipurpose herb. In tropical countries, it is often cultivated in homestead gardens and as a pot plant in many countries. Dried leaves of basil are used to flavour stew, sauces, salads, soups, meat and tea [15]. The plant has been considered ethnobotanically important because of its use in traditional health care system. Leaves and flowering parts of *O. basilicum* are traditionally used as antispasmodic, aromatic, carminative, digestive, galactogogue, stomachic, and tonic agents [16-18]. They have been also used as a folk remedy to treat various ailments such as; feverish illnesses, poor digestion, nausea, abdominal cramps, gastro-enteritis, migraine, insomnia, depression, gonorrhea, dysentery, and chronic diarrhea exhaustion [19]. Externally, they have been applied for the treatment of acne, loss of smell, insect stings, snake bites, and skin infections [20]. The plant has been widely used for its having antimicrobial, antibacterial, anti-viral, anti-fungal, larvicidal activity, anti-oxidant [25-31]. *O. basilicum* is also a globally important economic crop producing annually ca. 100 tones of essential oil worldwide and with a trade value as a pot herb of around US $ 15 million per year [32]. An immense study on the phytochemistry and pharmacology of *O. basilicum* was available on hand [33—36]. Many *in vitro* studies have been conducted on Lamiaceae species, including the genus *Ocimum*, using different explants, like nodal segments [37-41], shoot tip [42], leaf explants [43] and leaves derived calli mediated somatic embryogenesis [44], young inflorescence [45] and axillary buds [46,47]. Synthetic seeds were produced by encapsulating axillary vegetative buds harvested from garden-grown plants of these four *Ocimum* species in calcium alginate gel [48]. But there is no report on bioefficacy studies on in vitro derived calli of *Ocimum basilicum*. With this background the present study was aimed to screen the antibacterial potential of calli derived from inter-nodal and leaf segments of *Ocimum basilicum.*

EXPERIMENTAL SECTION

Callus induction

Inter-nodal and leaves segments of *Ocimum basilicum* L. 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 3 min followed by thorough washing with sterile distilled water. Surface sterilization was done with mercuric chloride solution (0.5 % w/v) for 2 min then washed thrice with sterile distilled water, the explants were cut into 0.7 cms in length and cultured on Murashige and Skoog [49] 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.). After few weeks, the *in vitro* proliferated callus cultures were sub-cultured onto MS medium supplemented with BAP, Kin, and NAA either alone or in combinations for organogenesis (Data's are not included). 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.

Preparation of extracts

In vitro derived callus (Leaves and inter – nodal segments) of *O. basilicum* were dried in the hot air oven and powdered using the electric homogenizer. The powdered samples were extracted with 150 ml of solvent (Aqueous, chloroform, benzene, Petroleum ether and ethanol) for 8 - 12 h by using the soxhlet apparatus.

Antibacterial Activity

The crude extracts of calli were concentrated and subjected for their antibacterial activity against the selected pathogenic bacteria. Stock cultures were made fresh every seven days on agar slants during this scheme of work. Pure bacterial cultures, namely The *Salmonella typhi, Klebsiella pneumoniae, Proteus vulgaris, Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated from clinical samples. The bacteria were identified and confirmed by conventional microbiology procedure. Stock cultures of *Salmonella typhi, Klebsiella pneumoniae, Proteus vulgaris, Staphylococcus aureus* and *Pseudomonas aeruginosa* were grown in nutrient broth at 30° C and were sub-cultured and maintained in nutrient broth at 4°C. Antibacterial study was carried out by disc diffusion method against the pathogens. Different concentrations of extracts ranging from 100 to 400 µl were used for bacterial sensitivity test. Aantibacterial activity was determined by measuring the diameter of the inhibition zone. The experiments were repeated in triplicate and the results were documented. Streptomycin was used as a positive control.

RESULTS AND DISCUSSION

Callus induction was observed on the inter-nodal and leaf segments on MS medium supplemented with 2, 4-D. Based on the concentration of plant growth hormone the callus formation frequency was varied and the results were tabulated in Table - 1. Highest percentage of callus formation (inter-nodal segments 75.2 ± 0.90; leaves segments 78.7 ± 0.51) was obtained on Murashige and Skoog's basal medium supplemented with 3% sucrose and 1.0 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 (Table - 1).

Five different solvent extracts of *in vitro* derived calli were examined for the antibacterial activity against the isolated human pathogens and the results are given in the table - 2. The maximum zone of inhibition 12 mm for *Salmonella typhi,* 13 mm for *Klebsiella pneumoniae,* 17 mm for *Proteus vulgaris,* 18 mm for *Pseudomonas aeruginosa* and *B. subtilis* and 10 mm for *Staphylococcus aureus* were observed. The ethanol extracts of leaf derived calli of *O. basilicum* illustrated the high degree of activity (5/6). The aqueous extracts of leaf derived calli of *O.*

basilicum demonstrated maximum zone of inhibition against the bacterium *K. pneumoniae* (13 mm). The ethanolic extracts of leaf derived calli of *O. basilicum* show the maximum activity compared to the stem derived calli of *O. basilicum* (Table -2).

Table 1: Effect of 2, 4-D on Callus production from the Leaves and Inter-nodal (Stem) segments of *Ocimum basilicum* L.

MS medium + Plant Growth Regulator (2, 4 – D) in mg/l	Mean percentage of callus induction ± S.E.		Type of Callus	
	Inter-nodal	Leaves	Leaves	Inter-nodal
0.0	00.0 ± 0.0	00.0 ± 0.0	NIL	NIL
0.5	57.4 ± 0.67	65.4 ± 0.46	Friable	Semi-friable
1.0	75.2 ± 0.90	78.7 ± 0. 51	Friable	Semi-friable
1.5	64.6 ± 0.52	73.2 ± 0.47	Friable	Semi-friable
2.0	56.4± 0.67	56.4 ± 0.62	Friable	Semi-friable
2.5	41.4 ± 0.58	44.8 ± 0.54	Semi-friable	Semi-friable

Table 2: Antibacterial activity of the Stem and Leaves derived calli extracts of *Ocimum basilicum*

Solvents	Conc. in µl	Zone of Inhibition (in mm)											
		K. pneumoniae		*S. aureus*		*P.vulgaris*		*P. aeruginosa*		*S. typhi*		*B. subtilis*	
		Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem
Ethanol	100	06	04	05	03	06	02	06	00	04	00	08	00
	200	08	06	07	05	11	03	11	00	07	04	10	05
	400	11	09	**10**	07	**17**	06	**18**	00	**12**	07	**18**	09
Chloroform	100	05	03	04	00	07	03	03	00	04	03	07	03
	200	07	05	06	03	12	06	07	00	06	07	12	05
	400	09	07	09	05	19	08	09	00	10	09	19	09
Petroleum ether	100	06	00	03	00	08	00	06	00	03	00	08	00
	200	09	00	05	00	12	00	11	00	05	00	12	00
	400	12	00	08	00	14	00	16	00	09	00	13	00
Aqueous	100	06	04	06	03	04	03	03	03	04	03	06	03
	200	09	06	07	02	07	05	05	07	08	05	09	07
	400	**13**	06	09	05	10	08	07	11	11	09	15	09
Benzene	100	05	00	05	00	06	00	03	00	03	00	05	00
	200	08	00	08	00	11	00	05	00	07	00	08	00
	400	10	00	08	00	13	00	08	00	09	00	12	**00**

The result of the present study revealed that the ethanolic extracts of leaves derived calli of *O. basilicum* can be used in the treatment of boils, sores and wounds, since *P. aeruginosa* have been implicated as causative agents of these diseases. Based on the present study it also confirmed that leaves derived calli of *O. basilicum*, can be used in the treatment of gastroenteritis, food borne illness, urinary tract infections, neonatal meningitis, nosocomial infections, wound, septicemia, pneumonias and from mild superficial skin infections to life-threatening systemic diseases, which are caused by *P. vulgaris* and / or *S. pyogenes.* The pathogen *S. typhi* is known to cause fever and food borne illness. In the present study the ethanolic extract of leaves derived calli of *O. basilicum* show the inhibitory activity against the bacterium *S. typhi* and thus the present study confirms the presence of active constituents present in these plants. Antibacterial properties of leaf derived calli are being increasingly reported from different parts of the world. The earlier observations on *Hypericum perforatum, Mimosa hamata* and *Baliospermum montanum* callus extract demonstrated significant antibacterial and antimicrobial activity [50, 51]. When the antibacterial efficacy of the leaves and leaf derived calli shows major difference. Thus the maximum zone of inhibition observed in the screening of four bacteria with five different extracts made from leaves of *O. basilicum* is only 14mm [52]. But in the present study the ethanolic extract of leaf derived calli of *O. basilicum* shows the maximum inhibition zone of

18mm among six different bacteria. The secondary metabolites like tannin, alkaloids, terpenoids, phenolics, coumarin and steroids as reported by Tomar et al [52] from ethanolic extract of leaves from *O. basilicum* may also be present in the *in vitro* derived calli at higher concentrations. The methods developed in this work make possible for the low volume and high potential production of active principles under *in vitro* condition in short duration with less amount of explants utilization. The present study will strengthen the phytochemical and antibacterial study on cell lines.

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