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In Vitro Propagation and Antibacterial Activity of *Ocimum basilicum* Linn.

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Abstract

The study introduced a simple, reliable procedure for the in vitro culture and standardization of indigenous plant *Ocimum basilicum.* A potential antibacterial activity was observed in the extracts (chloroform, methanol and petroleum ether) of *Ocimum basilicum* against selected bacterial strains (MTCC type strains) and their MIC (Minimal Inhibitory Concentration) values were 62.5 and 125 µg mL^{-1}. Existence of tannin, carbohydtares, phenolic compounds, proteins and aminoacids, anthraquinone glycosides, alkaloids were confirmed by phytochemical analysis. *In vitro* propagation from nodal explants and shoot tip of *O. basilicum* showed a higher level frequency of micro shoots on MS medium containing plant growth regulators at various combinations and concentrations. The nodal explants produced maximum shooting response (98 %); maximum shoot length (7.9 cm) and maximum multiple shoot formation (n=4.5) in BAP containing medium after 40 days. A 50% concentration of MS medium with combination of IBA and BAP showed maximum rooting response (78 %), maximum number of roots (n=12) and maximum length of root (8.67 cm) after 25 days. The regenerates were then hardened off and young plants were transferred to field. Nodal explants in MS medium with 2, 4 – D showed a fast growth and pale brown colored calli.

Keywords: Antibacterial Activity, MIC, Micropropagation, *Ocimum basilicum*

1. Introduction

The most important source of medicines are plants. *Ocimum basilicum* Linn., is fascinating in nature, hence the total part of plant is used as medicine for household treatment towards many human ailments [1]. Many studies prove that extracts of *O. basilicum* doesn't posses any antifungal activities but anti candidal and antibacterial effects [2]. It imparts strength and smoothness with certain perfume compounds mainly jasmine blends and hence used in preparation of scents. It also possesses insecticidal and insect repellent properties and effective against houseflies and mosquitoes. Besides its economic importance, it also has medicinal properties; which cures kidney problems, stomach associated ailments, headaches, coughs and shows bactericidal property against *Salmonella typhosa* [3], [4]. Anticancer activity and evaluation of fractions of *O. basilicum* extracts using human cancer cell lines were tested very recently [5].

High quality plant based medicines production is an important advantage of *in vitro* propagation. Conventional vegetative propagation has several limitations, which can overcome by micropropagation. Micro propagation has many advantages over conventional methods of vegetative propagation, which suffer from several limitations. Tissue culture plays an important role in genetic engineering process including gene transfer and development of transgenic plant. Simple extraction procedures and absence of significant amount of pigments make purification of compounds from tissue cultured plants which reduces the production and processing cost [6]. *Ocimum basilicum*

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flowering plants belonging to the family Lamiaceae. Previous knowledge on tissue culture of *Ocimum* is limited, but nodal explant or axillary shoot buds provide good source for obtaining large number of plants from single explant [7]. Very few reports are there on tissue culture of *O. basilicum*. When considering the pharmaceutical values of this plant, it is necessary to develop an efficient tissue culture method.

2. Materials and Method

2.1 Collection of Plants

The plant specimens (*O. basilicum* Linn.) were collected from Namakkal and Salem districts, Tamilnadu, India. Dr.R.Selvaraj, Professor in Botany, Annamalai University, Tamilnadu, India, has identified and confirmed the taxonomic characters of the plant.

2.2 Plant Extraction using Different Solvents

The leaves of *O. basilicum* were shade dried and later milled. The extract of each samples using solvents-methanol, chloroform and petroleum ether was carried out in soxhlet apparatus. The obtained plant extracts were then evaporated, dried and stored in sealed petriplates in refrigerator till use.

2.3 Bacterial Cultures

The bacterial cultures, *Bacillus subtilis* MTCC441, *Proteus mirabilis* MTCC742, *Staphylococcus aureus* MTCC796, *Salmonella typhi* MTCC733, *Pseudomonas aeruginosa* MTCC109, *Klebsiella pneumonia* MTCC741, *Escherichia coli* MTCC443 were purchased from Microbial Type Culture Collection Centre (MTCC), Chandigarh, India.

2.4 Antibacterial Activity of *O. basilicum*

About 1 mg of plant extracts was dissolved in 1 mL of Dimethyl sulfoxide (DMSO) and mixed well. The extract solution (100, 200, 300, 400 and 500 µL) was then added into sterile disc and allowed to dry. The bacterial cultures were swabbed into the Mueller Hinton agar (HiMedia, India) plates. After the inoculation, different plant extract discs were kept on the medium and incubated at 37 °C for 24 hrs. Antibacterial activity was determined by measuring the Inhibition zone.

2.5 Study of Minimal Inhibitory Concentration for Plant Extracts

About 1 gm of plant extracts was dissolved in 1 mL of DMSO and mixed well, then 1 mL of overnight culture was diluted serially as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} and 0.1 mL of inoculums from the 10^{-7} tube was taken to carry out MIC. To 1 mL of sterilized nutrient broth, varying concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.625 and 7.81 µL) of plant extracts were added in each test tube. 0.1 mL of overnight culture was then inoculated into tube and incubated at 37 °C for 24 hrs. Control tubes- antibiotic control and organism control were maintained.

2.6 Phytochemical Analysis of *O. basilicum*

Phytochemical analysis of different plant extracts (chloroform, methanol and petroleum ether) were carried out. The powdered (10 mg) plant extract dissolved in 10 mL DMSO and it was used as test solution. The chemicals such as alkaloids, flavanoids, carbohydrates, saponins, tannins and phenolic compounds [8], proteins and aminoacids [9], anthraquinone glycosides [10] were determined by the standard methods.

2.7 In vitro Propagation of *O. basilicum*

2.7.1 Shoot Induction and Elongation

Plant growth regulators like BAP, Kinetin, NAA and IBA at varying concentrations were used to standardize the most suitable concentration for shoot induction. The appropriate hormone, which promotes the maximum shoot induction, was used for subculture at regular intervals of 3 weeks and shoot elongation was then followed using appropriate medium. These were extended up to 40 days at 25 °C with illuminated light (16 hrs of light: 8 hrs of dark). During the experimental period, the average number of shoot and average shoot length were measured.

2.7.2 Root Induction

Growth regulators (NAA, IAA, IBA and BAP) at different concentrations and combinations in half strength MS media were used to evaluate their efficacy of rooting. The induced shoots were cut at the basal end and transferred to the medium under aseptic conditions. The cultures were incubated in the light at 25 °C for 20 days. The number

of roots and root length were measured (in cm) and the mean was calculated.

2.7.3 Hardening

Root induced plantlets from medium were carefully taken out and running tap water was used in order to washout medium residues and then treated with bavistin. The regenerates were shifted to soil in plastic cups (covered with plolythene bags) and kept in plant tissue culture lab. Young plants were transferred to field after 2 weeks and maintained at suitable conditions.

2.7.4 Callus Induction

Surface sterilized nodal explants were excised into pieces (1 cm) and were on the MS basal medium along with different auxins such as 2, 4-D and NAA and incubated under dark at 25 °C for 5 days. Then, they were transferred into light conditions and the percentage of callus induction was observed after 25 days period.

3. Results

3.1 Antibacterial Activity of *O. basilicum*

Disc diffusion method was carried out to analyse the antibacterial activity of different extracts (Chloroform, Methanol and Petroleum ether) of *O. basilicum* (Table 1). Methanol extracts showed maximum inhibition zone against *B. subtilis* and *S. aureus.*

3.2 Study of Minimal Inhibitory Concentration (MIC) for Plant Extracts of *O. basilicum*

Growth turbidity is only assessment for understanding minimal inhibitor concentration of various solvent extractions from *O. basilicum*. The MIC values of *O. basilicum* were evaluated and tabulated (Table 2). The petroleum ether, chloroform and methanol extracts of *O. basilicum* attained turbidity up to 125 µg mL^{-1} against *B. subtilis, P. mirabilis, S. aureus, P. aeruginosa* and *K. pneumoniae*. In the case of *S. typhi,* the MIC has been attained only from petroleum ether extracts at 125 µg mL^{-1}, whereas the concentration of 62.5 µg mL^{-1} for chloroform and methanol extracts. For *E. coli*, the MIC value reached up to 15.625 µg mL^{-1} from chloroform extracts and reached to 125 µg mL^{-1} from chloroform and methanol extracts of *O. basilicum* Chloroform extracts of *O. basilicum* showed the maximum MIC value.

3.3 Phytochemical Analysis

Analysis of Phytochemicals (Table 3) reveals the presence of carbohydrates and flavanoids and absence of tannin, phenolic compounds, protein and aminoacids, alkaloids, anthraquinone, glycosides and saponins in petroleum ether extract. Alkaloids, protein and aminoacids were present and saponins, carbohydrates, tannin and phenolic compounds, flavanoids and anthraquinones were absent in chloroform extract, whereas Methanolic extract showed the presence of alkaloids, protein and aminoacids, flavanoids, tannin and phenolic compounds and carbohydrates and absence of saponins and anthraquinone glycosides.

3.4 In vitro Propagation of *O. basilicum*

3.4.1 Shoot Induction and Elongation

Effect of various concentrations of plant growth regulators BAP, Kinetin, IBA and NAA on shoot induction of *O. basilicum* were studied (Table 4 and Figure 1) and among these growth regulators, maximum shooting response (98 %) and maximum shoot length (7.9 cm) was observed in BAP at a concentration of 3 mgL^{-1} and maximum multiple shoot formation (n = 4.50) in medium with BAP at 1 mg L^{-1}, Kinetin shows maximum response (89 %), maximum shoot length (4.75 cm) and maximum multiple shoots (n=1.57) at a concentration of 4 mg L^{-1}, NAA shows maximum response (89 %), maximum shoot length (4.3 cm) at 3mg L^{-1} and maximum multiple shoots (n=1.7) was observed in 2 mg L^{-1} of NAA. IBA shows maximum response (88 %) and maximum shoot length (4.11 cm) at 4 mg $L^{-1}$and maximum multiple shoots (n=2) was recorded at 3 mg L^{-1} of IBA concentration after 40 days (Plate 1).

3.4.2 Root Induction on *O. basilicum*

Maximum rooting response (78 %) and maximum number of roots (n=12) were observed in half strength MS medium with combination of 2 mg L^{-1} of IBA and 2 mg L^{-1} of BAP (Table 5 and Figure 2). Maximum root length (8.67 cm) was observed in combination of 3 mg L^{-1} of IBA and 2 mg L^{-1} of BAP after 25 days (Plate 2). It reveals that the hormones are highly inducing the growth at the significant level and earn the rich plant growth.

3.4.3 Hardening

Rooted plantlets from medium were then removed carefully and to remove excess medium, it was washed in running tap water and were given bavistin (fungicide)

treatment (1 g L^{-1}) for 10 min to protect the plant from any fungal attack. The regenerates were then transferred to soil in plastic cups (transparent polythene bags covered cups) and transferred into plant tissue culture lab. Young plants were transferred to field and maintained at suitable conditions after 2 weeks (Plate 3).

3.4.4 Callus Induction

Callus induction from the nodal explants of *O. basilicum* was observed. The callus was pale yellow and pale brown in colour (Plate 4). The explants were enlarged within 10 days of inoculation; however callus formation was started after 25 days. A maximum response of callus formation (92 %) was recorded in MS medium with 2 mg L^{-1} of 2, 4-D of auxine and minimum response of growth of callus in 5 mg L^{-1} of NAA (Table 6).

4. Discussion

In the present study, *in vitro* propagation of *Ocimum basilicum* by using various concentrations of plant hormones in M.S medium was carried out. Micropropagation has been found to be useful in the propagation of large number of medicinal plants. The *O. basilicum* popularly named as "Sweet basil" is widely used in Unani and Ayurvedic medicinal treatment [11]. It is a shrub of Asian origin which is small, perennial tropical [12]. In the present study nodal explants of *O. basilicum* were micro propagated in Murashige and Skoog medium with varying concentrations of different plant growth regulators. The maximum shoot formation in the present investigation was observed in MS medium with 3mg L^{-1} BAP. Maximum shoot proliferation was seen in MS medium fortified with BAP 4 mg L^{-1} + NAA 0.5mg L^{-1}. Medium with BAP (1 or 2 mg L^{-1}) at low concentration exhibits suitable for multiplication of shoot [13].

The maximum number of shoots (n=20) with (80 %) shoot response was observed on MS with (2.50 µM) TDZ [14]. The number of shoots obtained in the presence of $AgNO_3$ and PG was significantly higher (12.12 ± 0.33) than other media tested in *Vitex negundo* [15]. Our results showed maximum number of shoot formation in MS medium with 1 mg L^{-1} BAP. The maximum number of shoot buds in *Coleus vettiveroides Jacob,* were recorded on half strength MS medium containing 6.0 mg L^{-1} kinetin and 0.5 mg L^{-1} BAP at pH 5.8 [16].

For rooting of the micro shoots, 0.75 mg L^{-1} NAA exhibited results with 9.2 roots per shoot and 6.0 cm of average root length with an average of 95 % rooting response in half strength MS medium [17]. In this study, maximum root response (78 %) and maximum numbers of roots (15±0.51) was found in half-strength MS medium with 2 mg L^{-1} IBA + 2 mg L^{-1} BAP. Maximum root length was examined in 50% MS medium with 3 mg L^{-1} IBA + 2 mg L^{-1} BAP after 25 days. Mohammed (2010) [18] investigated the antimicrobial activity (disc diffusion method, methanol extracts) of six plant species which are used in Indian folklore medicine traditionally against bacterial and fungal infections caused by pathogens namely *S. aureus*, *S. epidermidis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *C. albicans* and *A. niger*.

Antibacterial activity of *O.basilicum* in different extracts were tested against *B. subtilis*, *P. aeruginosa, K. pneumonia, P. mirabilis*, *S. typhi*, *S. aureus,* and *E. coli* in the present study. Babu (2007) [19] explained that antibacterial activity of some Iranian plants are less in aqueous extracts when compared with that of solvent extracts which indicates that principle responsible for antibacterial activity is more soluble in organic solvents. Highest antibacterial activity was showed against *B. subtilis* by *A. nilotica* and *S. cordifolia* leaf extracts and significant activity against *Xanthomonas axonopodis pv. Malvacearum was* showed by *Z. mauritiana* leaf extract [20].

Sekar (2010) [22] reported that a better result was obtained in diethyl extracts of *Cassia auriculata* and *Emblica fischeri* to control bacteria. The antibacterial activity of methanolic extracts of *Abrus pulchellus* was tested against *S. aureus* MTCC902, *E. coli* MTCC405 and *P. aeruginosa* MTCC1934 by well diffusion method on agar plates. Syed (2011) [23] carried out antimicrobial activity of *Pterospermum diversifolium blume* was against *P.aeruginosa*, *E. coli, S. aureus* and *B. subtilis* by MIC and disc diffusion method. In the present study, MIC by broth dilution method of *O. basilicum* extracts includes methanol, chloroform, petroleum ether against *B. subtilis, P. mirabilis, K. pneumoniae S. typhi, E.coli, S. aureus and P. aeruginosa,* was done.

Prakash (2010) [24] studied preliminary phytochemical analysis of the *C. aromaticus* leaves which exhibited the presence of proteins, alkaloids, carbohydrates, flavanoids, glycosides, aminoacids, tannins, phenolic compounds and terpenoids [24]. In this study, preliminary phytochemical analysis of *O. basilicum* showed alkaloids, tannin and phenolic compounds, carbohydrates, proteins and amino acids, flavanoids, were present. Preliminary phytochemical analysis of *Withania somnifera* extracts showed the

presence of alkaloids, carbohydrates, glycosides, phenolic compounds, phytosterols, fixed oils and flavanoids [25].

5. Conclusion

This study gives enlightenment for plant tissue culture by providing a simple protocol for micro propagation of medicinally important plants. The study also shows the antibacterial efficiency of *O. basilicum*. Further studies can be carried out to develop efficient drugs from the bioactive compounds of medicinal plants against infectious diseases thus by overcoming the side effects of antibiotics.

6. References

1. Alia B., Nasreen J., Aji A., Saima N. B., Shahida H., Syeda H., "Phytochemical and pharmacological studies on *Ocimum basilicum* Linn- A Review", *Int J Cur Res Rev*, Vol. 4(23), pp. 73–83, 2012.
2. Ahmed A., Medine G., Meryem S., Hatice O., Fikrettin S., Isa K., "Antimicrobial effects of *Ocimum basilicum* (Labiatae) extract", *Turk J Biol*, Vol. 29, pp. 155–160, 2005.
3. Chopra R. N., Nayar S. L., Chopra I. C., "*Glossary of Indian Medicinal Plants* (Including the Supplement)", CSIR: New Delhi; 1994.
4. Faisal M., Shahzad A., Ahmad N., Anis M., Alatar A., Alwathnani A. H., "An efficient system for *in vitro* multiplication of *O.basilicum* through node culture", *Afr J Biotechnol,* Vol. 11(22), pp. 6055–6059, 2012.
5. Quamar K. A., Dar A., Siddiqui B. S., "Anticancer activity of *Ocimum basilicum* and the effect of ursolic acid on the cytoskeleton of MCF-7 human breast cancer cells", *Let in Drug Design and Discov*, Vol. 7(10), pp. 726–736, 2010.
6. Chang S. H., Ho C. K., Tsai J., "Micropropagation of *Taxus mairei* seedlings at different stages and recoverability of their palgitropic shoots", *Taiwan J Sci*, Vol. 13, pp. 29–39, 1998.
7. Pattnaik S., Chand P. K., "*In vitro* propagation of the medicinalherbs *Ocimu'm americanum* L. Syn. *O. canum* Sims. (Hoary basil) and *Ocimum sanctum* L. (holy basil)", *Plant Cell Rep,* Vol. 15, pp. 846–850, 1996.
8. Chandrashekar K., Santanu S., Prasanna K.," Phytochemical studies of aerial parts of the plant *Leucas lavandulaefolia*", *Der Pharma Chemica*, Vol. 2(5), pp. 434–437, 2010.
9. Kumar A. P., Kumud U., "Preliminary phytochemical screening and physico-chemical parameters of aerial parts of *Artemisia vulgaris*", *Int J Res Ayur Pharm,* Vol. 1(1), pp. 206–211, 2010.
10. Kokate C. K., Purohit A. P., Gokhale S.B., "Pathway to screen photochemical nature of nature drugs", *Pharmacognosy*, Vol. 42, pp. A3–A4, 2008.
11. Jain M. L., Jain S. R., "Therapeutic utility of *Ocimum basilicum* Linn. var. album", *Planta Med*, Vol. 54, pp. 66–70, 1988.
12. Dhar A. K., "Sweet Basil: *Ocimum basilicum*- a review", *J Med Arom Plant Sci*, Vol. 24, pp. 738–755, 2002.
13. Priyakumari I., Sheela V. L., "Micropropagation of *Gladiolus* cv. 'Peach blossom' through enhanced release of axillary buds", *J Trop Agri*, Vol. 43(1-2), pp. 47–50, 2005.
14. Najma I., Uzma R., Amla B., "Crucial role of TDZ in the quick regeneration of multiple shoots of *Clitoria Ternatea*", *Int J Pharma Sci Rev Res*, Vol. 6(2), pp. 23–26, 2011.
15. Mercy S., Sangeetha N., Doss G., "Phloroglucinol and silver nitrate enhances axillary shoot proliferation in nodal explants of *Vitex negundo* L. – an aromatic medicinal plant", *Iranian J Biotechnol*, Vol. 8(2), pp. 82–89, 2010.
16. Biju D., Arun K. D., Stalin N., "An efficient protocol for multiple shoot initiation in *Coleus vettiveroides* Jacob, medicinally important plant", *Adv Bio Tech*, Vol. 10(09), pp. 34–36, 2011.
17. Ashok K. J., Mudasir B., "*In-vitro* propagation of a medicinal plant *Portulaca grandiflora*. Hook", *World J Agri Sci*, Vol. 6(3), pp. 327–330, 2010.
18. Mohamed S. S., Hansi H. P. D., "Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants", *Int J Pharma Sci Res,* Vol. 1(10), pp. 430–434, 2010.
19. Babu S., Sathish S., Mohana D. C., Ragavendra M. P., Raveesha K. A., "Antibacterial evaluation and phytochemical analysis of some Iranian medicinal plant pathogenic *Xanthomonas pathovar*", *J Agri Tech,* Vol. 3(2), pp. 307–316, 2007.
20. Mahesh B., Satish S., "Antimicrobial activity of some important medicinal plant against plant human pathogens", *World J Agri Sci*, Vol. 4, pp. 839–843, 2008.
21. Sekar J., "Antibacterial activity of plant extracts- *Cassia auriculata* and *Emblica fischeri*", *Plant Articles,* Vol. 10(2), pp. 819–824, 2010.
22. Prashith K. T. R., Vinayaka K. S., Soumya K. V., Ashwini S. K., Kiran R., "Antibacterial and antifungal activity of methanolic extract of *Abrus pulchellus* wall and *Abrus precatorius* Linn. – A comparative study", *Int J Toxicol Pharm Res*, Vol. 2(1), pp. 26–29, 2010.
23. Syed H., Keshava C., Chandrashekar K. R., "Phytochemical evaluation and antibacterial activity of *Pterospermum diversifolium* blume", *Int J Pharma Sci,* Vol. 3(2), pp. 165–167, 2011.
24. Prakash O. R., Kedar K. R., Rabinarayan A., Sagar K. M., "Preliminary pharmacogonostical and phytochemical evaluation of *Coleus aromaticus* Benth. Leaf", *Int J Pharma World Res.* Vol. 1(4), pp. 1–19, 2010.
25. Shanthi M., Swaminathan C., "Evaluation of antibacterial activity and phytochemical analysis of leaves of *Withania somnifera* (L.) Dunal", *Int J Cur Res,* Vol. 3(3), pp. 010–012, 2011.