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In vitro Clonal propagation of an Important Medicinal Plant Ocimum sanctum and assessment of its antimicrobial & phytochemical activities

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ABSTRACT

Plants have aided human kind as sources of medicinal agents since its primitive beginnings. *Ocimum sanctum* is an aromatic plant belonging to the family Lamiaceae. An effective protocol for *in-vitro* micro propagation of holy basil (*Ocimum sanctum* L.) from nodal explants was stabilised. Among the various cytokinins evaluated for shoot development and proliferation BAP was most effective. MS medium supplemented with (0.5-1.0mg/L) BAP in combination with (0.2-0.3mg/L) NAA was observed to be ideal for shoot elongation and multiplication. Rooting was established with NAA at concentrations of 0.5 µM which considerably increased rooting percentage, root length, and root number. Fully rooted plantlets were transferred to poly trays containing sand, vermiculite and soil (1:1:2) and covered with transparent plastic bags to prevent loss of humidity and acclimatized for a period of 3 weeks. After primary hardening the plantlets were transferred to a greenhouse with simulated habitat for better survival. Phytochemical screening of the plant leaves shows the presence of saponins, alkaloids, flavonoids, cardiac glycosides, steroids, phenols and tannins. Antimicrobial activities of different extracts of leaves of Ocimum sanctum were studied by disc diffusion method against Gram-positive and Gram-negative bacterial pathogens. The chloroform extracts exhibited wide range of antibacterial activity where as methanol extract showed slightly lower activity against bacteria.

Key words- *Ocimum sanctum,* antimicrobial activity, *Escherichia coli, Bacilus subtilus.,* Phytochemical analysis.

Introduction

Medicinal plants are a valuable source of compounds for the pharmaceutical industry and traditional medicine. Although synthetic drugs and antibiotics are prerquisites for current medical practice, plants provide a major aid to the pharmaceutical industry (Fowler, 1983;

Sahoo et al., 1997). *Ocimum sanctum*, commonly known as "Holy Basil", belongs to the family of Lamiaceae. The plant is held sacred by Hindus all over the world as it is an herb that is used for religious purposes, in addition to its great medicinal values (Banu and Bari, 2007). Diverse medicinal properties of *O. sanctum* for example antidiabetic (Mukherjee *et al.*, 2006), antioxidant (Samson *et al*., 2007), cardioprotection (Sood *et al.*, 2006), antifungal (Awuah and Ellis, 2002), immunostimulant (Mukherjee *et al*., 2006) have attracted entrepreneurs to set eyes on this plant. The industry sector perceives *O. sanctum* not just as a spice consumed in household kitchen. They also see it as a potential lead to the production of drugs that are important to answer the insatiable needs of the population. *Ocimum sanctum* is one of the most sacred herbs of India, and is an integral part of ancient Hindu traditions. According to Hindu mythology, *Ocimum sanctum* originated as one of the 14 Ratnas (gems or treasures) from the ocean as the ultimate sacred plant to enhance health and remove diseases. *Ocimum sanctum* is believed to be a manifestation of the Goddess Lakshmi, the wife of God Vishnu, and is worshiped for health, wealth, and happy married life [19]. The plant is treated as a blessing from God, a religious symbol, and a magic herb as reported in many ancient medicinal texts such as Ayurveda, Siddha and Unani [10].

The major difficulty in the use of Lamiaceae species for pharmaceutical purposes is its individual variability, due to genetic and biochemical heterogenicity (Shetty, 1997. Viera et al, 2001. Dode et al, 2003). The conventional method of propagating this species is through seeds, but seed viability is very poor and low germination limits its multiplication. Moreover, progenies derived from seeds are not true to type, due to cross-pollination (Heywood, 1978). *In- vitro* culture techniques offer an option for the study and conservation of rare, threatened or endangered medicinal plants (Mauseth, 1979; Ajithkumar and Seeni, 1998; Sahoo and Chand, 1998; Prakash et al., 1999), and a tool for efficient and rapid multiplication of species when high uniformity of progeny is required. *In vitro* studies have been conducted on Lamiaceae species, including the Ocimun genus, using different explants, like nodal segments [1][3][22], leaf explants [17], young inflorescence [23] and axillary buds [4]. In this work we established a reliable plantlet regeneration protocol using nodal explants for large-scale production of *Ocimum sanctum* .There is increasing interest in using these techniques for rapid and large-scale propagation of medicinal and aromatic plants (Bajaj et al.,

1988.Vincent et al. 1992. Krishnan and Seeni, 1994. Purohit et al. 1994; Sudha and Seeni, 1994. Bhat et al. 1995. Patnaik and Debata, 1996. Sahoo et al., 1997).

Although the whole plant is medicinally important, the leaves are usually the most common form of treatment, and are either used raw or steeped in hot water. The medicinal properties of *Ocimum sanctum* leaves include: antimicrobial, anticancer, antistress, adaptogenic, stimulant, expectorant, nervine, antipyretic and antiperiodic [10]. These beneficial properties have led to *Ocimum sanctum* being named "Queen of Herbs", "Incomparable One" and "The Mother Medicine of Nature" and one of the most valued medicinal and religious herbs in India [24].

MATERIALS AND METHODS

Ocimum sanctum plants were collected from Green House, Tectona Biotech Resource Centre, Bhubaneswar. Shoot tips (nodal segments of 0.8-1.0 cm) with inactive auxiliary buds of about 0.6 cm were removed from plants and used for *in vitro* propagation studies. Selected nodal segments were straightaway washed in running tap water followed by washing with 10% Tween 20 (10 min) and 1 % (w/v) Bavistin for 5 minutes. The explants were then sterilised for 20 seconds with 70% ethanol, followed by 0.1% (w/v) $HgCl_2$ for 2-3 minutes and rinsed 5-7 times in sterile distilled water inside laminar air flow chamber. Subsequently, explants were chopped at the cut ends and inoculated.

Culture Medium and Conditions

Shoot tips were cultured on modified MS basal medium containing 3% (w/v) sucrose for callus initiation. The pH of the medium was adjusted to 5.7 with 1 N NaOH or 1 N HCl prior to gelling with 0.8% (w/v) agar. Horizontally implanted explants in bottles were maintained at 25 ± 2°C temperature, uninterrupted light with 16 hours photoperiod at 50 µmol light intensity by cool white fluorescent tubes with 60-70% relative humidity.

Callus induction

Two week old sub cultured shoot buds were transferred on to modified MS medium augmented with 2, 4 - D (0.5mg/l) and varying concentrations of Kinetin (0.25mg/l –1mg/l)

for optimization of callus.

Shoot bud initiation

For shoot bud initiation nodal segments of *O. sanctum* were inoculated on MS media supplemented with different concentrations of BAP (0.5 mg/l -1.0mg/L) and Kinetin (Kn 1.0 mg/l -3.0 mg/l) .

Multiple shoot initiation

Fully matured shoots of around 3 - 4 cm in height were cut at their nodal segments and transferred to MS full strength medium (supplemented with Kn 1.0+NAA 0.5) for initiation of multiple shoots

***In Vitro* rooting**

Elongated microshoots recovered from nodal explant cultures had no roots. Hence, for rhizogenesis, 3-4cm long shoots were separated as minicutting, cultured on MS medium supplemented with auxins. In most of the experimental protocols, half strength media was the best choice for root induction, but here, an attempt was made to replicate the same results using a full strength medium. Roots were visible within 25-35 days following the transfer of elongated shoots to the rooting media. Of all the concentrations tried for rooting on *in vitro* raised shoots, the best results were obtained in IBA at 1.0 mg/l (8 roots per explant). The highest root length i.e. 3.1 cm was encountered at IAA 1mg/l + IBA 2 mg/l respectively.

The explants showing root initiation after 8-10 days of starting the culture were transferred to medium of the same composition to promote further proliferation (with the increase of number of root laterals, length of roots etc.) with the lapse of 25 days. The results indicate that IBA concentrations supplied to MS medium significantly influenced root proliferation and shoot growth. The full strength of MS medium without any PGR failed to induce rooting of regenerated shoots. Another infrequent combination of auxin and cytokinin i.e BAP + NAA was incorporated to check for any root formation. Results did show root development but the incubation time was found to be more (around 4 weeks) as compared to

other formulations. Prolific rooting of *in vitro* grown micro shoots is critical for the successful establishment of these shoots in the greenhouse and field.

Acclimatization of Regenerated Plants

Fully rooted plantlets of nearly 5 to 6 cm in length were extracted from the culture medium and washed under running sterile water to remove agar. The plantlets were transferred to poly trays containing a mixture of sand, vermiculite and soil (1:1:2) and covered with transparent plastic bags to prevent loss of humidity. The set-up was maintained at 26 ± 1°C, 80 – 85% relative humidity and at a light intensity of 50 µ mol m-2 s-2 under a 16 h photoperiod in culture room conditions and acclimatized for a period of 3 weeks. After primary hardening the plantlets were transferred to a greenhouse with simulated habitat for improved survival.

Phytochemical & Antimicrobial Activity Assesment

Young leaves of *Ocimum sanctum* were collected from Green House, Tectona Biotech Resource Centre, Bhubaneswar. The leaves were washed thoroughly with normal tap water followed by washing with sterile distilled water. Leaves were dried under shade at room temperature and ground to powder using an electric blender. The powder was stored at 4°c in light air tight container. The air-dried powdered leaf material (in 20 g lots) of *O. sanctum* was extracted with 200 ml volumes of solvents- methanol, chloroform and distilled water, separately at 4°c, in succession. Solvent residuals from combined extracts were evaporated by a vacuum rotary evaporator. For hot extraction in a soxhlet apparatus, 20 g of powder-mass was placed in the extractor and a volume of 200 ml of a solvent was used during 6 h of soxhlation, till colourless extracts precipitated in the extractor. After filtration, each extract was concentrated by means of evaporation. The resultant sticky-mass was dried in hot air oven; the solid mass was stored in a sufficient volume of 10% dimethyl sulphoxide (DMSO) with a drop of Tween-20. Cold and hot methanol extracts of *O.sanctum* were pale green to thick greenish in colour. Aqueous extracts were green in colour and sticky after the concentration. The stock concentration of each extract was retained at 30 mg/ml, for further use.

Phytochemical Screening (Qualitative analysis):

Chemical tests were carried out using an aqueous extract to identify various constitutes employing standard methods of Sofowara, Trease and Evaans and Harbone.

Tests for Tannins:

About 2 ml of the aqueous extract was stirred up with 2 ml of distilled water and few drops of $FeCl^3$ Solution were added. Development of a green precipitate was indication of presence of tannins.

Tests for Saponins:

5 ml of aqueous extract was shaken vigorously with 5 ml of distilled water in a test tube and heated. The formation of stable foam was accepted as an indication of the presence of saponins.

Test for phlobatannins:

About 2 ml of aqueous extract was added to 2 ml of 1% HCL and the mixture was boiled. Accumulation of red precipitate was accepted as an evidence for the presence of phlobatannins.

Tests for Flavonoids:

To 1 ml of aqueous extract, 1 ml of 10% lead acetate solution was added. The development of a yellow precipitate was taken as a positive test for flavonoids.

Test for Terpenoids:

2ml of organic extract was dispersed in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and warmed for about 2 min. Formation of a greyish colour reveals the presence of terpenoids.

Test for glycosides:

Liebermann's test: 2ml of the organic extract was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added to it. The solution was cooled well on ice. Sulphuric acid was then added carefully, a colour change from violet to blue green indicates the presence of a steroidal nucleus (that is, a glycone portion of glycsoside).

Test for steroids:

- Formation of red colour in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform with addition of 2 ml concentrated sulphuric acid, reveals the presence of steroids.

- Formation of a greenish colour when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acid indicates the presence of steroids.

Antimicrobial Activity Assesment

Test Organisms: Prior to sensitivity testing, each of the bacterial strains were cultured onto Nutrient agar plate and incubated for 18 to 24 hours at 37˚C. A single colony was then cultured in 25 ml LB Broth for 24 hours at 37˚C.

Disc Diffusion Method:

Disc diffusion assay for antimicrobial susceptibility test was carried out according to the standard method by Bauer *et al.* (1966) to assess the presence of antibacterial activities in the plant extracts. A bacterial culture was used to lawn Muller Hinton agar plates uniformly using a sterile cotton swab. The plates were dried out for 15 minutes and then used for the sensitivity test. The sterile discs impregnated with a series of plant extracts were placed on the MH agar surface. Each test plate comprised of six discs- one positive control, which is a standard commercial antibiotic disc, and five treated discs. The standard antibiotic discs were Ampicillin (20 µg/ml). The negative control was DMSO (100%). Besides the controls, each plate had three treated discs placed at equidistance to each other. The plate was then incubated at 37°C for 18 to 24 hours depending on the species of bacteria used in the test. After the incubation, the plates were checked for occurrence of inhibition zone. The inhibition zones were then measured using callipers and observations recorded. The tests were repeated in triplicates to ensure reliability.

Results and Discussion

Ocimum sanctum L., plants were effectively regenerated from nodal explants from field-grown young plants , on MS medium augmented with 0.5- 3 mg/l BAP & 0.5- 2.5 mg/l Kinetin for multiple shoot induction. The multiple shoot induction response with respect to the test concentrations of growth hormones is presented in Table 2. Of the two combinations tested in the present study, Kinetin with NAA exhibited highest shooting rate and better shoot length per explants. MS media augmented with Kn 0.5 mg/l + IAA 1.5 mg/l and Kn 0.5 mg/l

+ 1.5 mg/l of NAA gave 84.4% and 86.7% shoots respectively. On the other hand, MS media supplemented with IAA (Kn1.5 mg/l + IAA 0.5 mg/l) showed 71% shooting with less number of shoots (2.24) and shorter shoot lengths (1.97cm) per explants. MS media supplemented with Kn 0.5 mg/l and NAA 1.5 mg/l generated 5.96 shoots per explants whereas multiple shoot induction on MS media supplemented with 0.5 mg/l of kinetin with 1.5 mg/l of IAA showed 3.58 shoots. Average number of shoots on MS medium supplemented with NAA was found to be significantly higher ($P < 0.5$) when compared to the MS media supplemented with IAA. On the other hand, shoot lengths appeared to be almost similar in the two combinations of growth hormones tested in the present study. Overall, MS media supplemented with 1 mg/L of kinetin with 0.5 mg/l of NAA showed highest multiple shoots (95.5%), maximum number of shoots (7.33) and even relatively longer shoots (4.81 cm) per explants (four weeks after ideal cultural conditions). This response was significantly better ($P < 0.05$) than that from other combinations tested in the present investigation. From the results, it is evident that the higher proportion of kinetin with half the concentration of NAA holds good for attaining rapid multiple shoots under *in vitro* conditions. Kinetin with different root induction growth hormones (auxin types and concentrations) greatly affects auxillary shoot regeneration from nodal explants. Medium without growth regulator (control) gave no regeneration response and explants swelled and became necrotic two weeks after inoculation. Multiple shoot production, number of shoots and length of the multiple shoots per explants significantly reduced in all combinations of growth hormones tested either above or below optimized concentrations. Growth response (reduction in number of shoots per each node) at higher or lower than optimal concentration of cytokine has also been reported in several medicinal plants [26-28]. Other cytokinins like Benzyl adenine have been reported to overcome apical dominance, release of lateral buds from dormancy and promote shoot formation in dicot plants [29]. Effect of BA on multiple shoot formation of *in vitro* propagated *O. bascilium* has also been reported [11,12].

In general, for the preservation of valuable medicinal plant resources, micro propagation through axillary bud proliferation has been proven to be a handy tool [18][8]. Studies by [6] and [20] have revealed that the type of plant growth hormone and its dosage influenced the frequency of shoot formation. [2] used cytokinins in the media to stimulate axillary or

adventitious shoot development. He found that the type and concentration of cytokinin had far reaching effects on shoot multiplication.

Phytochemical analysis :

The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms (Shadomy *et al*., 1985; Odds,1989). In general agreement with previously published qualitative analysis of Lamiaceae species (Shan *et al*., 2005), the phytochemical screening of the phytoconstituents of the plant studied showed that leaves were found to have flavonoids, saponins, cardiac glycosides, alkaloids, phenols and tannins. They were known to show medicinal activities well as exhibiting physiological activity (Sofowara, 1993). This is also in agreement with Javanmardi and his co others, who reported that rosmarinic acid is the most abundant component in *O. Basilicum* (Javanmardi *et al*., 2002). *Ocimum* species has been extensively reported for its essential oil content (Roberto *et al*., 2003). Phenolic compounds are a significant group of secondary metabolites, which are synthesized by plants due to plant adaptation in response to biotic and abiotic stresses (Infection, water stress, cold stress, high visible light) (Pitchersky and Gang, 2001). So far, in the *Ocimum* species the *O. basilicum* and *O. sanctum* leaves have been reported for their secondary metabolite content (Javanmardi *et al*., 2002; Hakkim *et al*., 2007).

The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities (19). The phytochemical characteristics of the leaf extract of *Ocimum sanctum* investigated are summarized in Table 2. The results reveal the presence of medicinally active constituents like tannins, alkaloids, terpenoids, steroids, saponins and flavanoids, phlobatannins, glycosides in the leaves of *Ocimum sanctum*.

The alkaloids contained in plants are used in medicine as anaesthetic agents (20). The presence of saponins in plants has been reported to be responsible for the toning and stimulating activities observed in Chinese and Japanese medical herbs. The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive

constituents responsible for the efficacy of the leaves of the plants studied. The occurrence of some of these compounds has also been confirmed to have antimicrobial activity. Hence it could be inferred that the plant extracts could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection.

Antimicrobial efficacies of plant extract:

Antibacterial activity assay of plant extract against Gram negative (*E.coli)* and Gram positive (*Staphylococcus aureus and Bacillus subtilis)* bacteria revealed an antibacterial activity against the test microorganisms. The zone of inhibition of plant extract against Gram negative and Gram positive bacteria was measured. The results indicated that Ocimum *sanctum* leaf extract showed effective antibacterial activity both in Gram negative and Gram positive bacteria. The antimicrobial effect of the plant extract was examined using the disc diffusion assay which is mainly used to test the sensitivity of bacterial strains towards antibiotics where a clear zone around the discs reflects the bacterial sensitivity towards antibiotics. The mean diameters of inhibition zones obtained in the present study are given in Table (4). The results showed that *Ocimum sanctum* leaves extract showed good inhibition against the three studied bacterial strains. This observed antimicrobial activity could be explained by the fact that plant extract may attach to the surface of the cell membrane upsetting permeability and respiratory functions of the cell. The interaction of plant extract with microbial cytoplasmic components and nucleic acids can inhibit the respiratory chain enzymes, and interferes with the membrane permeability, limiting the development of bacteria. It is also likely that extract not only interact with the surface of membrane, but can also percolate inside the bacteria. The susceptibility of Gram positive and Gram negative bacteria to the plant extract was found to vary from one study to another. According to *Nagajyothi and Lee*, plant extract were found to be significantly toxic against the fungal and gram positive microbes and exhibited mild toxicity against *E.coli*. Whereas *Antony et al.*, reported that extract had a considerably minimal antimicrobial activity on Gram positive bacteria compared to Gram negative bacteria which they attributed to the high lipo polysaccharide and thick peptidoglycan layer of the microorganisms. Our results revealed that leaves extract exerted nearly similar antibacterial activity against both Gram positive and Gram negative bacteria.

Conclusion:

In conclusion, an effective protocol for micro propagation of an important medicinal plant, *O.sanctum*, was developed in this study by testing various concentrations of growth regulators and culture conditions. The results will make the conservation and propagation of the species much easier. The presence of phytochemicals in the investigated medicinal plant would be responsible for the antimicrobial activity of the extracts. Leaves of *Ocimum sanctum* extracted in methanol, chloroform and water were evaluated for phyto constitutes present in them. These leaves contain phytochemicals like alkaloids, tannins, saponins, terpenoids, glycosides, phlobatannins, anthraquinones and steroids. The literature indicates that the antibacterial activity is due to different chemical agents present in the extract, including essential oils (especially thymol), flavonoids and triterpenoids and other natural phenolic compounds or free hydroxyl groups. Methanol extracts of *Ocimum sanctum* posseses antimicrobial potential against both gram positive and gram negative bacteria. Plant extracts are quite efficient in the control of several diseases caused by microorganisms. It is therefore confirmed as a useful antimicrobial agent. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. It is quite safer to use as an herbal medicine as compared to chemically synthesized drugs.

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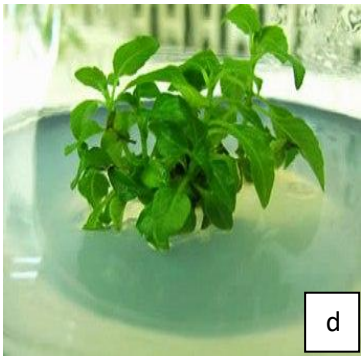

Figure 1: (a) Mother Plant (b&c) Shoot proliferation from nodal explant (d) Shoot multiplication on MS + Kn (e) Elongated shoot (f) Rooted plantlets transferred to pot

MS + growth regulators (mg/l)		Percent of calli development (Mean ± S.E)*		Av. No. of shoot buds per culture (Mean ± S.E)*	
BAP	**Kn**	**A**	**B**	**A**	**B**
0	0	0	0	0	0
1.0	0	8.36±0.7 a	12.6±0.8 a	1.3±0.5 a	2.2±0.3 b
1.5	0	12.5±0.8 b	18.5±0.7 c	1.6±0.6 c	2.1±0.5 b
2.0	0	20.2±0.6 e	24.2±0.8 e	1.4±0.4 b	2.3±0.4 b
2.5	0	20.8±0.8 e	26.8±0.4 f	1.4±0.6 b	2.6±0.7 b, c
3.0	0	20.5±0.9 e	21.6±0.7 d	2.3±0.8 d	3.2±0.2 c, d
0	1.0	11.8±0.6 b	14.2±0.8 b	1.8±0.4 c	2.4±0.3 b
0	2.0	12.6±0.9 b	17.6±0.5 c	2.2±0.4 d	2.9±0.6 c
0	3.0	14.4±0.5 c	18.8±0.4 c	2.4±0.6 d	2.5±0.4 b

Table 1: Effect of different concentrations of cytokinins and auxins on shoot bud regeneration from leaf explants of *Ocimum sanctum* after 4 weeks (A) and 6 weeks (B) of subculture

2.0	0	30.2±0.8 f	32.2±0.7 g	3.5±0.5 e	3.8±0.2 e
2.0	0	42.7±1.0 g	45.5±0.9 h	3.6±0.3 e	4.6±0.5 f
0	2.0	20.6±0.8 e	26.8±1.0 f	2.8±0.5 d	3.4±0.2 d
0	2.0	22.2±0.7 e	26.6±0.8 f	3.2±0.7 e	3.4±0.3 d
2.0	0	52.8±1.2 h	62.7±1.0 i	6.8±0.6 f	8.0±0.6 g
2.0	0	55.6±1.0 i	74.2±0.8 j	8.7±0.3 g	10.7±0.4 h
2.0	0	12.2±0.7 b	12.8±0.6 a	1.2±05 a	1.6±0.5 a
2.0	0	18.8±0.4 d	20.2±0.5 d	1.7±0.4 c	2.5±0.3 b

***Mean of 15 replicates / treatment; repeated thrice**
Mean followed by different letters are significantly different at the 5 % level

Table 2:-Effect of growth regulators on Root Formation of *Ocimum sanctum*

Hormonal Treatments (Root induction media)	Concentration of PGR (mg/L)	No. of days for rhizogenesis	No. of roots	Root length (in cm)	Remarks
R_0(without hormone)	-	-	-	-	No rooting response
R_1 (IAA)	1.0	18 days	5	1.5	++ Thin roots
R_2 (IBA)	1.0	20days	9	2.3	++ Thick roots
R_3 (IAA+IBA)	1.0+2.0	20 days	6	3.1	++ Thick roots
R_4 (BAP+NAA)	2.0+0.4	30 days	4	0.9	+++ Thick & well rooted

Table 3: Phytochemical constituents of the leaf extract of *Ocimum sanctum* leaves

Chemical Constituent	Methanol extract	Chloroform extract	Aqueous extract
Tannins	++	+	+
Saponins	+	+	-
Phlobatannins	+++	++	+
Flavanoids	++	+++	+
Terpenoids	+++	-	+
Glycosides	++	+++	-
Steroids	+++	+++	++
Alkaloids	++	++	+
Anthraquinones	-	-	-

Key: negative (-) low (+) moderate (++) high (+++)

Table 4: Size of inhibition zone of leaf extract with different solvents of O*cimum sanctum* against different bacteria*:*

Name of the Organism	Zone of inhibition (in mm)				
	DMSO (100µl)	Ampicillin (100µl)	Chloroform (100µl)	Methanol (100µl)	Aqueous (100µl)
E.Coli	0	14	7	11	4
B.subtilus	0	12	9	12	4
S.aureus	0	11	9	10	3