

Research Article

In-vitro* Micropropagation and antimicrobial activity of *Chrysanthemum indicum

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Abstract: Plants have been an important source of medicine for thousands of years. The World Health Organization estimates that up to 80% of people use plants which has traditional remedies such as herbs. *Chrysanthemum indicum* is an important medicinal plant spreading widely in most part of the world, belonging to the Family- Compositae. Almost all of its parts are used in Ayurvedic and modern systems of medicine, leaves and flowers are the most important part in the field of medicine and drug. In the present study, *in vitro* regeneration and antimicrobial activity of leaf extracts were carried out. The plant was found to possess antibacterial activity against some of the pathogens.

Keywords: *Chrysanthemum indicum*, Ethanol, Leaf extract, Growth regulators, antimicrobial activity.

INTRODUCTION

Plants have always been a major component of traditional system of healing in developing countries, which have also been an integral part of their history and culture. Medicinal plants offer alternative remedies with tremendous opportunities. Many traditional healing herbs and plant parts have been shown to have medicinal value especially in the rural areas and these can be used to prevent and cure several human diseases. Even today, majority of the world population depends on herbal health care practice [1, 2].

The strategic importance of reviving indigenous medical practices to provide safe and affordable primary healthcare to the people of the world is now recognized. During the last two decades or so, WHO's Health Assembly has passed a number of resolutions in response to this resurgence of interest in the study and use of traditional medicines and in recognition of the importance of medicinal plants to health care of people in many developing countries [1].

Chrysanthemum indicum Linn is traditionally reported for the having larvicidal activity [3, 4], Anti-arthritis [5], anti-inflammatory and immunomodulatory [6] and hepatoprotective activity [7]. The aerial parts (stem, leaves and flowers) of *Chrysanthemum indicum* to treat hypertensive symptoms and several infectious diseases, such as fever and stomatitis [8].

Its flowers are also commonly used as tea to treat some eye disease [9] The plant extracts possess central and peripheral analgesic properties, lowering blood pressure, also exhibited inhibitory activity against microbes [10]. The tea prepared from *C.indicum* flower could prevent sore throat and promote reduction of fever. The flower extract have antioxidant activities and DNA damage preventive capacity[11].

An investigation on standardising media composition for *in-vitro* micropropagation and antimicrobial activity of leaf extracts was done using protocols.

MATERIALS AND METHODS

***In vitro* Regeneration**

The present investigation on *in vitro* micro propagation of *Chrysanthemum indicum* was attempted in the plant tissue laboratory of the PG and Research Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore.

Source of plant materials

Chrysanthemum indicum plants were collected from six to eight years old plants growing in Tiruppur district, and used for the entire course of study.

Explants selection and mode of sterilization

The explants namely node, internode, leaf were harvested from *in vivo* plants, and washed thoroughly in running tap water and teepol. It is then treated with ten percent (w/v) of Methyl-3-benzimidazole carbamate (Bavistin) solution. The explants were subsequently surface sterilized with 0.1 % (w/v) mercuric chloride solution for 7-10 minutes and washed 3-4 times in sterile distilled water. The surface sterilized explants were trimmed gently with the help of sterile surgical blade and aseptically inoculated on pre-cooled medium.

Culture media preparation and Growth regulators employed

Murashige & Skoog (MS) basal medium were employed in present study. The composition of the medium is given below. The pH of the medium was adjusted to 5.8-5.9 prior to the addition of agar.

Three important groups of growth regulators such as Auxins, Cytokinins and Gibberillic acid (GA3), growth adjuvant like activated charcoal were used in the

experiments. All the growth regulators and growth adjuvant are stored at 4°C until use. Three auxins namely α -Naphthalene Acetic acid (NAA), 2,4 - Dichlorophenoxy Acetic Acid (2,4 -D) and Indole 3-Acetic Acid (IAA) were used in these experiments.

Explant Inoculation

The explants were taken from the cultures maintained in laboratory conditions in the growth room. For multiplication experiments, shoot tips, nodes, internodes and leaves were used as explants. For root induction experiments, shoot tips and nodes were used as explant. Inoculation was done in the laminar air flow chamber. The chamber was wiped with alcohol and cotton, the forceps and scalpel were kept in the steripot and UV light was put on for 15minutes before starting the inoculation. After 15minutes, the UV was put off and visible light and airflow was put on. Both arms were wiped with alcohol. The bottles containing media and explants were also wiped with alcohol and cotton and kept in the work desk of the chamber. The bottles were opened and cultures picked out carefully with forceps. They were kept on the brown paper on the work desk. Shoot tips, nodes, internodes and leaves were cut out carefully with the scalpel by holding with the forceps. It was then inoculated in the bottles containing medium using scalpel.

Antimicrobial Activity

Sampling of the plant material

Fresh leaves of *Chrysanthemum indicum* were collected. The leaves were washed thoroughly 2-3 times with running tap water and then air dried under shade. The total dried mass was grounded to a fine uniform powder. The powder obtained after grinding was kept in small plastic bags with proper labelling.

Ethanollic extraction of plant material

For extraction, the disease free and fresh plants were selected. About 10g of fresh and healthy leaves were taken and surface sterilized with 0.1% mercuric chloride for 20 seconds. Again the leaves were washed thoroughly with distilled water. With the help of mortar and pestle, the leaves were grounded well using ethanol as the solvent. It was then subjected to centrifugation for 15min at 10000rpm. Again it was filtered through Whatmann No.1 filter paper. The supernatant were collected and the plant extracts of different dilution.

Bacterial inoculum preparation

Bacterial cultures used in this study were obtained from Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore. Bacterial cultures included in this study were *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Psuedomonas aeruginosa*

and *Streptococcus mutans*. All the cultures were grown in Nutrient broth medium. The inoculum was used for antibacterial assay.

Fungal inoculum preparation

The cultures were obtained from Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore. It includes *Trichoderma viridae*, *Candida albicans*, *Penicillium chrysogenum*, *Aspergillus niger*. The fungal cultures were subcultured and maintained on Sabourauds dextrose medium.

Agar well diffusion method

The antimicrobial activity was tested against ethanolic extract of *Chrysanthemum indicum*. About 15-20 ml of Nutrient agar medium was poured in the sterilized petridish and allowed to solidify. One drop of bacterial strains was spread over the medium by a sterile cotton swab. Similarly, 15 – 20ml of Sabouraud Dextrose agar medium was prepared and fungal culture was spread over the medium by sterile cotton swab. Wells of 6mm in diameter and about 20mm was punctured in the culture medium using sterile cork borer. Varying concentrations of the extract (20 μ l, 40 μ l, 60 μ l, 80 μ l, 100 μ l, 120 μ l) was added to the wells. Plates were incubated at 37°C for 24 hours in case of bacteria and at room temperature for 3 -4 days for fungal cultures. Antimicrobial activities were evaluated by measuring the diameters of zone inhibition after the incubation period.

RESULTS AND DISCUSSION

In-Vitro Regeneration

Bulging of explant and induction of callus was observed on 10th day of culture. Callus formation was obtained within a period of 2 weeks in media supplemented with IAA and BAP. Callus obtained after incubation can be further proliferated and maintained by subculturing in auxin rich medium. Regeneration of shoots and roots from calli was obtained by subculturing on MS medium containing 2, 4 - D.

Antimicrobial Activity

The antimicrobial activity of *Chrysanthemum indicum* was analyzed. The bacterial culture of *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli* in petriplates incubated along with the extract were checked for growth inhibition zone of organisms. The antimicrobial activity of the plant extract and their efficiency was quantitatively assessed using well diffusion methods. After 24 hours, the antibacterial activity of crude extract and ethanolic extracts of the plant *Chrysanthemum indicum* was studied and listed (Table.1).

Table 1: Antibacterial activity of ethanolic leaf extract of *Chrysanthemum indicum*

MICROORGANISMS	Zone of inhibition (mm) at different concentrations						
	Control	20 µl	40 µl	60 µl	80 µl	100µl	120µl
<i>Klebsiella pneumonia</i>	--	14	15	15	18	20	21
<i>Bacillus subtilis</i>	--	12	12	13	14	15	15
<i>Psuedomonas aeruginosa</i>	--	12	12	13	13	13	15
<i>Staphylococcus aureus</i>	--	8	8	9	8	10	10
<i>Escherichia coli</i>	--	11	12	15	17	17	19
<i>Streptococcus mutans</i>	--	12	12	13	14	15	15

The ethanolic extracts of *Chrysanthemum indicum* showed highest degree of inhibition against *Klebsiella pneumonia* and *Escherichia coli*, moderate degree of inhibition against *Streptococcus mutans*, *Psuedomonas aeruginosa*, *Bacillus subtilis* and minimum degree of inhibition against *Staphylococcus aureus*.

The fungal culture of *Trichoderma viridae*, *Candida albicans*, *Penicillium chrysogenum*, *Aspergillus niger* in petriplates were incubated along with the extract were checked for growth inhibitions zone of organisms. After 3 days of incubation, it was observed that *Candida utilis* showed highest degree of inhibition and no zone was observed in the other 3 plates (Table 2).

Table 2: Antifungal activity of ethanolic leaf extract of *Chrysanthemum indicum*

MICROORGANISMS	Zone of Inhibition (mm) at Different Concentrations						
	Control	20 µl	40 µl	60 µl	80 µl	100µl	120µl
<i>Candida utilis</i>	--	15	18	21	24	25	28
<i>Penicillium chrysogenum</i>	--	--	--	--	--	--	--
<i>Trichoderma viridae</i>	--	--	--	--	--	--	--
<i>Aspergillus niger</i>	--	--	--	--	--	--	--

CONCLUSION

From the present study it is inferred that the plant has potent antimicrobial activity. Further investigation on the isolation of bioactive components from *Chrysanthemum indicum* would hold to increase its potential to use the plant as the source of new drugs. This study shows that *Chrysanthemum indicum* is a potential herbal medicine.

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