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Comparative Study on the Antibacterial Potential of Leaf And Leaf-Derived Callus Extracts of Simarouba Glauca DC

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ABSTRACT

Background: *Simarouba glauca*, an exotic species, is a plant known for its significant medicinal properties.

Aim: The study showed the antibacterial activity of leaf-derived callus extract and leaf extract of *Simarouba glauca*. The objective is to develop a faster and more reliable *in-vitro* callus induction method for *S. glauca* using leaves as explants, and to compare the antibacterial activity of leaf and callus extracts.

Method: Callus induction was evaluated using MS medium supplemented with different growth regulators. MS medium with Different hormonal combinations was used for the *in vitro* induction of callus. The antibacterial activity of Petroleum ether, Chloroform, and Acetone extracts of leaf and callus extracts of *S. glauca* was tested against the five different bacterial strains. The bacterial strains tested include *B. subtilis, S. typhi, S. aureus, E. coli*, and *V. cholera*.

Result: MS medium with 2,4-D (5 mg/L) proved best callus induction for leaf explants, yielding maximum fresh and dry weights. Maximum zone of inhibition was observed in the Petroleum ether leaf extract against *the E. Coli* bacterial strain, followed by the acetone leaf extract against *E. coli*. In the case of antibacterial activity of callus extract, Acetone showed maximum zone of inhibition against *E. coli* strain, followed by Petroleum ether callus extract against *B. subtilis*.

Conclusion: This study successfully developed an effective method for in-vitro callus induction in *S. glauca* and confirmed the antibacterial potential of both leaf and callus extracts.

Keywords: *Simarouba glauca*, in-vitro callus induction, antibacterial activity, plant extracts.

INTRODUCTION

According to the World Health Organization, 80% of the global population primarily relies on traditional medicine for health care (Fakim., 2006). People have believed in the healing powers of plants since ancient times. Plant-derived substances have recently garnered significant interest due to their diverse applications (Baris *et al*., 2006). Out of the several thousand medicinal plant species around the globe, only a small portion have been investigated both phytochemically and pharmacologically (Hostettmann, 1999).

Traditionally used medicinal plants have recently caught the attention of the biological science community. This involves isolating and identifying secondary metabolites from plants and using them as active ingredients in medicinal preparations (Taylor *et al*.,2001).

Biotechnological approaches, particularly plant tissue cultures, offer promising alternatives for producing valuable medicinal compounds from plants. These methods supplement traditional agriculture in the large-scale production of bioactive plant metabolites (Rao *et al*., 2000). Research in plant tissue culture has developed in the large-scale production of secondary metabolites, which are helpful in the pharmaceutical industries. Large-scale plant tissue culture is an attractive alternative to traditional plantation methods, as it provides a controlled supply of biochemicals that is independent of plant availability (Sajc *et al*., 2000).

The Simaroubaceae family consists of 32 genera and over 170 species of trees and shrubs with a pantropical distribution. It is notable for its bitter compounds, which contribute to its pharmaceutical properties (Fernando and Quinn, 1992; Muhammad *et al*., 2004). Since 1930, the Simaroubaceae family has been the subject of many studies regarding its chemical constitution, and numerous compounds have been isolated and their structure has been elucidated; among these include quassinoids, alkaloids, triterpenes, steroids, coumarins, anthraquinones, flavonoids, and other metabolites (Barbosa *et al*., 2011). Quassinoids can be considered a taxonomic marker of the Simaroubaceae family since it is the most abundant group of natural substances and their synthesis is almost exclusive (Saraiva *et al*., 2006; Almeida *et al.,* 2007). Studies also claim the antitumor, antileukemic, and antifeedant activities of quassinoids in *Ailanthus excelsa of Simaroubaceae* (Lavhale and Mishra, 2007).

Simarouba glauca is an exotic species belonging to the family Simaroubaceae. It is commonly found in North America, and it is also known as Paradise tree, Aceituno, or American bitter wood. It is widely used for the treatment of cancer; hence it is known as the tree of solace for cancer. It is commonly known as a Laxmitaru in India and is known for its high medicinal value. It is a native of El Salvador that has been introduced in India during 1960 (Mikawlrawng *et al*., 2014). It was first introduced in India in 1966 from Brazil for its edible oil Aceituno or Acetone oil under the plant introduction scheme of the Indian Council of Agricultural Research (ICAR), New Delhi, in the National Bureau of Plant Genetic Resources (NBPGR) Station at Amravati, Maharashtra, and in Dr. Panjabrao Deshmukh Agricultural University, Akola, in 1970.

MATERIALS AND METHOD

Plant Material

Young seedlings of *S. glauca* were planted in the Botanical Garden of Mercy College. Small young twigs with disease-free leaves were collected from one-year-old S. *S. glauca*. Leaves were removed from the branches. Both the leaves and nodes were placed in a small conical flask and rinsed with running tap water thoroughly for 30 minutes, and then washed in Tween 20 for 15 minutes. Then the explants were washed with distilled water.

The explants were disinfected with 70% alcohol and washed for 60 seconds. Moreover, the surface was subsequently sterilized with 0.1% Mercuric chloride solution for 5 minutes. Moreover, it was followed by repeated rinsing with sterilized distilled water 3 times, under aseptic conditions. The explants were cut into 1-2 cm size, inside a laminar air flow chamber, and inoculated into the medium and incubated in laboratory conditions.

Callus Induction

The medium used for inducing callus was Murashige and Skoog (MS) medium. Stock solutions of micro

and macro nutrients, and growth regulators were prepared in distilled water and stored in a refrigerator. Calcium chloride was dissolved separately in distilled water and then added to the rest of the solution to avoid precipitation during media preparation. The hormones added were BAP, IAA, IBA, and 2,4-D. They were added in various combinations of concentrations to initiate the callus phase. MS medium without any plant growth regulators is used as a control. The pH of the medium was adjusted to 5.6. Agar was added and boiled to dissolve the agar. The medium was distributed to culture tubes and plugged with cotton. The culture medium was sterilized in an autoclave at 121˚c at 15lbs for 20 minutes and kept under aseptic conditions for inoculation. The leaves of *S. glauca* were cut into required sizes and inoculated onto MS medium. Details regarding callus formation were observed, and results were recorded.

The callus formed in different hormonal combinations was collected, and its fresh weight was noted. The calli was air-dried under laboratory conditions, and its dry weight was noted. The powdered callus was used for the extraction process. It was initially extracted with petroleum ether for 48 hours using a magnetic stirrer. After extraction, the mixture was filtered, and the resulting extract was stored in the refrigerator. Subsequently, the callus was extracted with chloroform and acetone.

Soxhlet extraction

15g of the powdered leaf was used for Soxhlet extraction. Different solvents were used for the extraction based on their increasing order of polarity. The powdered leaves were first extracted in 300 mL of petroleum ether for 72 hrs. Then the extracts were collected, filtered, and stored in a refrigerator for further studies. After that, chloroform and acetone were subsequently used for the extraction process.

Test Organisms

The test organisms were collected from the culture collection of Microbial Technology (IMTECH), Chandigarh, India. The bacterial strains tested were *B. subtilis, E. coli, S. aureus, S. typhi, and V. cholera*. The bacterial strains were sub cultured on nutrient agar slants and incubated at room temperature.

Culture Media and Preparation of Inoculum

28g of nutrient agar was weighed and dissolved in 1000ml of distilled water. The medium was autoclaved at 121˚c at 15 lbs for 20 minutes, and 15 ml of media was poured into sterile petri dishes under aseptic conditions. The plates were allowed to solidify.

Antibacterial Activity

The method used follows the protocol established by Barry and Thornsberry (1991). Nutrient agar was employed for antibacterial screening studies. The bacterial strains were spread on the medium using a cotton swab under aseptic conditions. Blank sterile discs (Hi-Media Laboratory Private Ltd., Mumbai) of 6mm were used for the study. The discs were impregnated with 500µg of the leaf and callus extracts of different solvents. Pure solvents were used as the negative control for the study. Streptomycin discs (30µg) of 6mm were used as a positive control. It was used to compare the diameter of the inhibition zone. The discs were placed carefully, and the zone of inhibition was calculated by measuring the diameter of the zone in mm after 24 and 48 hours. The plates were incubated at 37°C. The experiments were repeated three times, and the values were statistically analyzed with the determination of standard deviation.

RESULT AND DISCUSSION

In-vitro callus induction

The study demonstrated the antibacterial activity of the callus and leaf extract of *Simarouba glauca*. In vitro regeneration analyses were conducted to evaluate the potential for developing a faster and more reliable method for callus induction in *Simarouba*. Different hormonal combinations were used for the *in*

vitro induction of callus. MS + 2, 4-D (5 mg/l) proved best for the early induction of callus in leaf, which also produced maximum fresh weight and dry weight. It was followed by 0.5 BAP and 0.5 IAA. Callus induction was observed after 11 days of inoculation using MS + 2, 4-D at a concentration of 5 mg/l.
In the case of node MS + 2, 4-D (5 mg/l) was ineffective for early callus induction. MS + 0.5 BAP and 0.5 IAA produced the highest fresh and dry weights in nodal explants. Callus induction and growth were observed to be lower in nodal explants. Initially, the callus was white, but it later changed to yellowish-green and brown.

Antibacterial Activity

The antibacterial activity of Petroleum ether, Chloroform, and Acetone extracts of leaf and callus extracts of *S. glauca* was tested against the five different bacterial strains. The bacterial strains tested include *B. subtilis, S. typhi, S. aureus, E. coli*, and *V. cholera*. The result showed that the maximum zone of inhibition was noted in Petroleum ether leaf extract against *E. coli* bacterial strain, followed by acetone leaf extract against *E. coli*.
In the case of antibacterial activity of callus extract, Acetone showed maximum zone of inhibition against *E. coli* strain, followed by Petroleum ether callus extract against *B. subtilis*. The antibacterial activity was found to be higher in the leaf extracts than in the callus extract. Leaf and callus extract of Acetone was only found to be resistant against *V. cholera*. Solvents do not exhibit any antibacterial activity, as no zone of inhibition was observed. The zone of inhibition decreased after 48 hours. However, the antibacterial activity against *S. aureus* was found to be increased after 48 hrs. The callus extracts in chloroform and acetone showed an increased zone of inhibition in the presence of antibiotics.

Table- 1. Different hormone combinations and no: of days required for callus induction in leaf explants

Sl. no	BAP (mg/l)	IAA (mg/l)	2,4D (mg/l)	IBA (mg/l)	Days required for callus induction	Growth up to 40 days from inoculation of explant
1	0.5	0.5	-	-	17	+++
2	4	0.5	-	-	12	++
3	-	-	5	-	11	++++
4	2	1	-	-	12	++
5	-	-	-	1	19	+

Table-2. Fresh weight and dry weight of callus induced in leaf explants using different hormonal combinations

Hormones used (mg/l)	Fresh weight(g)	Dry weight(g)
0.5 BAP+ 0.5 IAA	0.481	0.127
2 BAP+ 1 IAA	0.416	0.124
4 BAP+0.5 IAA	0.371	0.093
5 2, 4 D	6.442	0.857
1 IBA	0.271	0.067

Table-3. Antibacterial activity of crude leaf extract of *Simarouba glauca* against 5 bacterial strains after 24 hours (inhibition zone in mm ± SD)

Solvents	***B. subtilis***	***S. aureus***	***E. coli***	***S. typhi***	***V. cholera***
Petroleum ether	22±0.001	19±0.002	28±0.010	17±0.012	_
Chloroform	23±0.003	18±0.010	24±0.001	18±0.011	_
Acetone	21±0.012	25±0.003	27±0.012	19±0.002	19±0.011
Solvent	_	_	_	_	_
Streptomycin	22±0.001	21±0.012	24±0.002	22±0.003	20±0.02

Table-4. Antibacterial activity of callus extract of *Simarouba glauca* against 5 bacterial strains after 24 hours (inhibition zone in mm ± SD)

Solvents	***B. subtilis***	***S. aureus***	***E. coli***	***S. typhi***	***V. cholera***
Petroleum ether	15±0.021	14±0.011	13±0.001	_	_
Chloroform	_	11±0.001	14±0.012	_	_
Acetone	14±0.013	-	18±0.010	12±0.011	14±0.012
Streptomycin	15±0.011	16±0.012	23±0.013	20±0.011	18±0.012

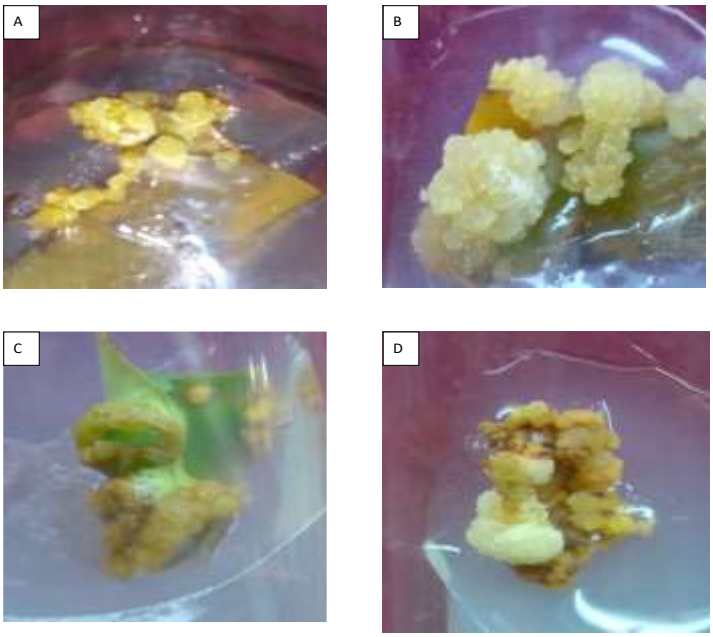

Figure 1. Stages of callus proliferation from leaf explants of *S. glauca*. (A). Callus induced from leaf explants supplemented with 0.5 mg/l BAP+ 0.5mg/l IAA. (B). Callus induced from leaf explants supplemented with 5mg/l 2- 4, D. (C). Callus induction in 2mg/l BAP+ 1mg/l IAA. (D). Callus sub cultured in the MS medium supplemented with 5mg/l 2- 4, D .

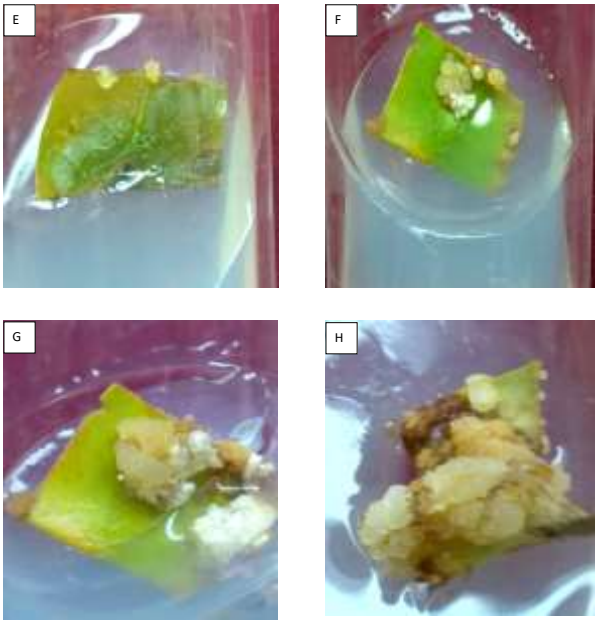

Figure 2. Stages of callus induction in MS media supplemented with 5mg/l 2- 4, D. (E). Callus induction after 10 days of inoculation. (F). Callus proliferation after 18 days. (G). Callus development after 25 days. (H). Callus proliferation after 40 days of inoculation.

Conflict of Interest

The authors declared no potential conflict of interest.

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