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Production of An Indole Alkaloid from Callus Cultures of *Salvadora Persica* L.

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ABSTRACT

Callus cultures derived from cotyledon explants of *Salvadora persica* L.were established on MS medium. Culture conditions as well as hormonal manipulation have been standardized for initiation of callus cultures. Optimum callus cultures were established on MS medium augmented with various levels of auxins and kinetin. Addition of ascorbic acid and proline at lower concentration significantly reduced browning of initiated callus as well as enhanced production of metabolites. An indole alkaloid salvadoricine known to occur in leaves have also been isolated from undifferentiated tissues of *S. persica*. Isolated compound was confirmed by spectral analysis.

Key words: *Salvadora persica*, *indole alkaloid*, *callus cultures*, *salvadoricine*.

INTRODUCTION

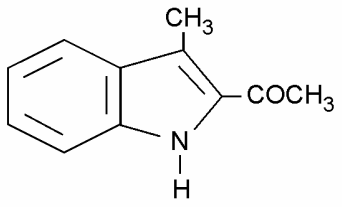
Alkaloids are naturally occurring organic bases which are universally found in higher plants with pronounced physiological functions. Amongst all, indole alkaloids are emerging as the most fascinating class of alkaloids because of their great diversity in molecular structure and biosynthesis.

An indole alkaloid Salvadoricine have been extracted from a medicinal plant of arid zone i.e. *Salvadora persica*. The plant derived its name from the persian name i.e. Darakht-i-miswak or toothbrush from the fact that the wood is much employed for the manufacture of tooth brushes and toothbrush made up of it strengthen the gums, keep them away from becoming spongy and improve digestion[1]. Salvadoricine (2-acetyl,3-methyl indole) has been found to be effective against various dental diseases, scurvy, rheumatism, etc.

Moreover, it also shows antibacterial activity against several pathogenic bacteria, particularly cariogenic bacteria of teeth[2].Moreover, chemical analysis of *S. persica* revealed that it contains a total of 19 natural substances which are beneficial for dental health. Its natural antiseptics have a bactericidal action killing harmful micro-organisms in the mouth. Its tannic acid content has astringent qualities which protects the gums from diseases and its aromatic oil increases salivation.

Recently, *S. persica* has been used in the commercial manufacture of toothpastes in Egypt, India, Pakistan, Switzerland and also in the United Kingdom.

Plant tissue culture technique,emerged as a viable tool for the production of various drugs such as paclitaxel (Taxol), provides a model for the production of anticancer agents from woody plants[3-5]. Here, we report the factors affecting callus initiation and growth as well as salvadoricine production in developing callus of *S. persica*.



Salnadoricine ($C_{11}H_{11}NO$)

MATERIALS AND METHODS

Plant Material

Seeds of *S. persica* were procured from JNV University, Jodhpur and were surface sterilized with 0.1% $HgCl_2$ for 10-15 minutes, thoroughly rinsed sterile distilled water and germinated on ½ MS medium without any hormones as well as in pots.

Induction and Initiation of Callus Cultures

Cotyledon explants obtained from *in vitro* germinated seedlings were cultured on MS medium[6] with varying concentration of 2,4-D (0.5-5.0 mg/l) and BAP/Kn (0.1-1.0 mg/l). Whereas leaf explants excised from *in vivo* seedlings were surface sterilized and after regular sterilization procedure cultured on to MS medium supplemented with auxins and various concentrations of cytokinins. Callus cultures thus raised were transferred to fresh medium once in every three weeks. Manipulation of various hormonal regimes as well as sucrose concentration was also optimized. The cultures were maintained under fluorescent light with 12 hr photoperiod. The pH of the medium was adjusted to 5.8 with 1N NaOH and 1N HCl before autoclaving. Sucrose levels varying from 10 to 80 mg/l in the maintenance medium were applied to study the sucrose effects. At the same time,effect of various additives i.e. ascorbic acid, proline, glutamic acid was also determined.

Extraction and Determination of Indole Alkaloid

Alkaloids are soluble in water and could be extracted with alcohol. Callus tissue was homogenized and Soxhlet extracted with ethanol (3-4 hr) filter and concentrated with the solvent under reduced pressure (in vacuo) Residue was subjected to acid-base extraction.

Acid-Base Extraction

Residue was mixed with 1% H_2SO_4 (3-4 ml) and then added few ml. of ammonia solution drop by drop to make pH 9-10.

Resulting crude alkaloid mixture was extracted with ethyl acetate (3-4 hrs). Then ethyl acetate soluble fraction was concentrated and subjected to preparative TLC (Silica gel) with a solvent system (Benzene: Chloroform (1:9) and observed under ultraviolet light.

RESULTS AND DISCUSSION

Effect of plant growth regulators on callus induction

Plant growth regulators are known to influence both growth of the callus as well as production of metabolite in the culture media[7-9]. Callus cultures of *S.persica* were established on MS medium supplemented with auxins and cytokinins. Cotyledon produced callus on MS medium supplemented with 2,4-D(2.5mg/l) and Kn(0.1mg/l) (Table-1).Whereas, leaf explants produced callus on MS medium having 2,4D (3.0 mg/l) + IAA (0.5 mg/l) + BAP (0.5 mg/l). However, callus produced from leaf explants was compact and less in amount. It was noticed that concentration and combination of various phytohormones considerably influenced the growth and metabolite production in *S. persica*.

Nahalka et al 1996 and Akcam-Oluk et al 2003[10,11] reported enhanced production of alkaloid in cell suspensions of Drosophyllum and *Catharanthus roseus* respectively when combination of NAA and BAP was used in the medium.

Table-1:Effect of different sugars on biomass and alkaloid production:

Sugars (30 gm/l)	Biomass (gm DCW^{-1})	Alkaloid (Salvadoricine) (mg g^{-1} DCW)
Glucose	7.34 ± 0.05	3.70 ± 0.06
Fructose	4.06 ± 0.07	2.16 ± 0.14
Sucrose	17.36 ± 0.14	2 ± 1.7
Lactose	3.28 ± 0.7	2.16 ± 0.41
Glycerol	3.00 ± 0.02	1.54 ± 0.14

*Cultures harvested after 21 days of incubation, biomass was measured as gram dry cell wt (g DCW 1^{-1}) and alkaloid in mg g^{-1} dry cell wt (mg g^{-1} DCW).

Effect of Sugar Concentration and Type

Sucrose concentration play a pivotle role in induction of callus cultures as well as affect secondary metabolic production in plant cell cultures, some nodule cultures and compact callus aggregates[12-15]. Manipulation of concentration as well as type of sugars affect biomass as well as alkaloid production (Table 1). Out of various sugars, glucose, sucrose showed significant effect on biomass and alkaloid production. Whereas lactose and glycerol showed poor growth with low metabolite accumulation. Sucrose concentration at 30 mg/l enhanced both biomass level as well as alkaloid production (Table- 2). However its higher concentration decreased biomass as

well as alkaloid content. The optimal sucrose concentration for alkaloid and biomass production was found to be 40-50 gm/l. At this concentration 40-50 of the total alkaloids to be released into the medium.This is in agreement with the reports of earlier workers[5,14,16].

Effect of additives

Produced callus was brown and meager in amount. Addition of ascorbic acid and proline at lower concentration (10-30mg/l) enhanced both metabolite production as well as amount of callus. At the same time, it also reduced browning of initiated callus.

Table-2: Effects of sucrose concentration on characteristics of the callus cultures:

Sucrose Concentration (g/l)	Dry Biomass	Total Alkaloids in Cells (mg g^{-1}) DW	Alkaloids in the Medium (mg l^{-1})	Alkaloid Yield (mg l^{-1})
10	12.1 ± 0.8	0.33 ± 0.05	7.34 ± 0.05	12.9 ± 1.1
20	12.9 ± 1.2	0.42 ± 0.11	7.36 ± 0.14	14.0 ± 1.4
30	14.6 ± 1.2	0.99 ± 0.10	16.5 ± 1.4	31 ± 2.0
40	13.6 ± 1.2	0.89 ± 0.08	10.8 ± 0.9	22 ± 1.7
50	10.8 ± 0.9	0.44 ± 0.10	7.34 ± 0.05	15.8 ± 1.3
60	4.3 ± 0.41	0.30 ± 0.12	7.12 ± 0.18	14.6 ± 1.2
70	3.9 ± 0.31	0.20 ± 0.10	3.90 ± 0.07	4.3 ± 0.39
80	3.6 ± 0.32	0.18 ± 0.10	3.85 ± 0.06	3.9 ± 0.31

*Cultures were maintained on MS medium + 2,4D (2.5 mg/l), Kn (0.1 mg/l) + proline(20 mg/l) + ascorbic acid (40mg/l) and data collected on day 25 of culture. Data were expressed as mean ± SD (n=3), DW = Dry Wt).

Qualitative analysis

When TLC plates were observed under ultraviolet light, white, crystalline and shiny spots were seen. Furthermore, plates were sprayed with CAS(Cerric ammonium sulfate) reagent, subsequently, activated at 100°C for 10 minutes. Then again observed under ultraviolet light. The spots developed were prominently fluorescent white, crystalline and shiny.

These spots were eluted with methanol and then crystallized. It's mp was noticed at 143-144°C. The purified material was subjected to spectral analysis.

IR Spectra

It's IR spectra showed the following peaks:

- > N-H stretching 3270 cm^{-1}
- > C-O stretching 1620 cm^{-1}
- > CH stretching (in $–CH_3$ group) 2860 cm^{-1}
- > CH stretching (in aromatic system) 3120 cm^{-1}
- > CH stretching (in $–CH_3$) 1405 cm^{-1}
- C-C stretching 1075 cm^{-1}
- C-H stretching (in $COCH_3$ group) 2930 cm^{-1}

Thus, the appearance of the bond at 3270 cm^{-1}, 1620 cm^{-1}, 2860 cm^{-1}, 2930 cm^{-1} showed the presence of >NH group, >C=O, $–CH_3$ and $–COCH_3$ group in the compound. The peaks at 3120 cm^{-1}, 1075 cm^{-1} supported the presence of aromatic ring in the compound (Fig.-2).

UV Spectra

Its UV spectrum showed absorption maxima at λ_{max} 264 nm with maximum absorbance (10 Abs), which in characteristic for acyl-indole chromophore[17-19] (Fig.1).

EI mass spectrum

Its EI mass spectrum indicated the molecular ion peak at m/z 173 (90%). Exact mass measurement of the molecular ion peak afforded the mass 173.8410 which was calculated 173.8406 for $C_{11}H_{11N}O$.The base peak

occurring at m/z 158 (100%) indicated the loss of methyl group while the peak at 130 (78%) corresponded to M^+–$COCH_3$ [19].

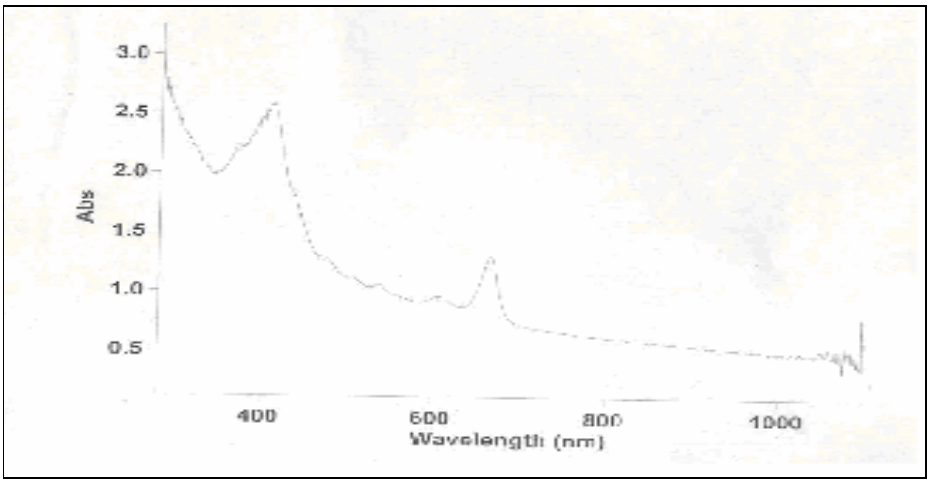


Fig-1: UV Spectra

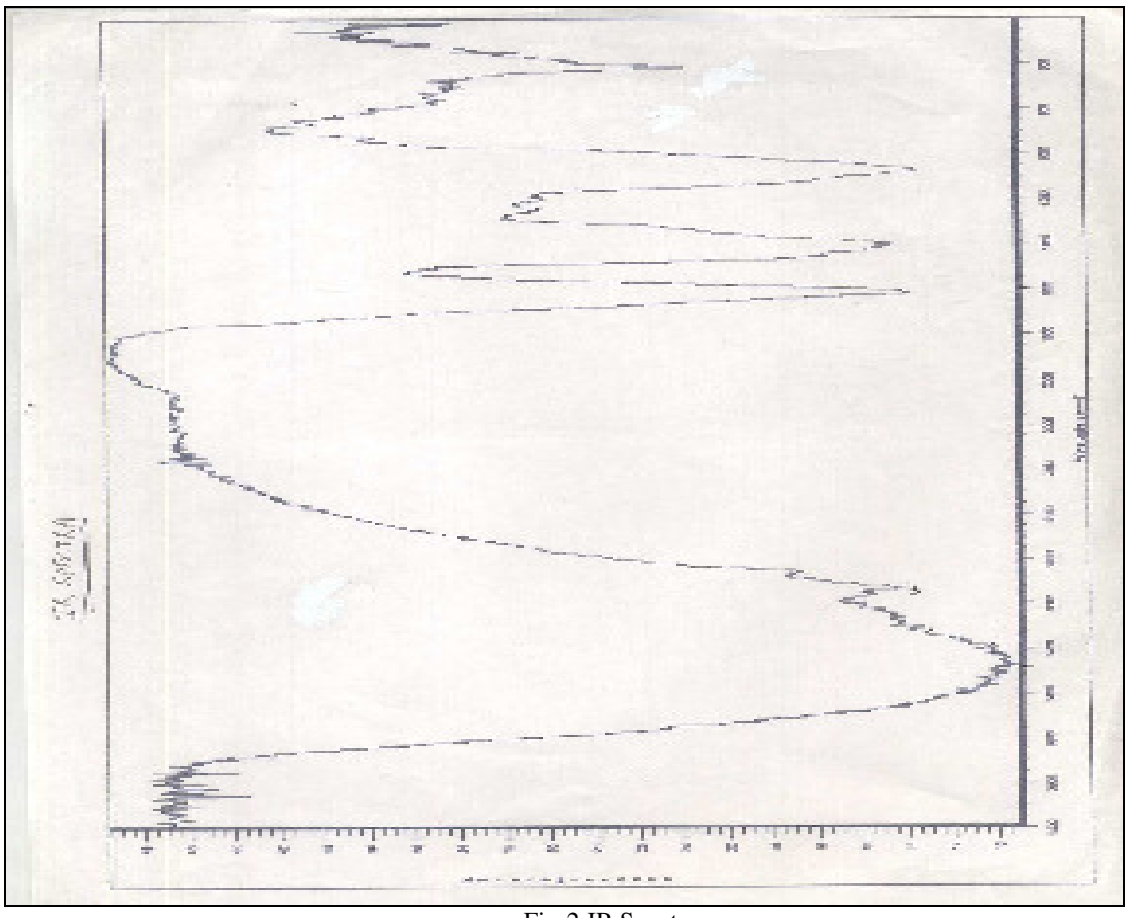


Fig-2:IR Spectra

Table-3: Chemical shift values of Salvadoricine

Carbon No.	Chemical Shift	Carbon No.	Chemical Shift
2	130.2	8	110.4
3	119.6	9	130.7
4	125.6	2 –CH_3	13.5
5	123.2	$COCH_3$	27.8
6	118.2	$COCH_3$	189.2
7	122.3		

NMR Spectra

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz, δ ppm) indicated the presence of eleven protons. Two sharp singlet each integrating for three protons resonating at 2.58 and 2.68, were assigned to 3-methyl and 2-acetyl protons, respectively. The aromatic protons (7.08-7.69) also supported the position (C-2) of acetyl group[20,21]. Each of these protons were identified individually, C-5H (7.69,dd, $J_{5,6}$ = 8.1 Hz, $J_{5,7}$ = 1.1 Hz), C-6H (7.29, ddd, $J_{6,5}$ = $J_{6,7}$ = 8.1 Hz, $J_{6,8}$ = 1.1 Hz), C-7H (7.08, ddd, $J_{7,6}$ = $J_{7,8}$ = 8.1 Hz, $J_{7,5}$ = 1.1 Hz) and C-8H (7.46, dd, $J_{8,7}$ = 8.1 Hz, $J_{8,6}$ = 1.1 Hz).

Similarly, downfield chemical shift of indolic N-H (8.80) also supported the 2-acetyl substitution. Since in 3-acetyl-2 methyl indole this indole proton was found to resonate at 8.38. At the same time, ^{13}C-NMR ($CDCl_3$, 75 MHz,δppm) chemical shift values of indole alkaloid (Table-3) were found similar with earlier reports[18,20].

CONCLUSION

The aim of the present study was to standardize and optimize culture conditions for enhanced production of salvadoricine *in vitro*. Phytohormones, carbon source, additives influenced the biomass and salvadoricine production in *S. persica*. Activity of isolated compound was tested on various fungi and bacteria.

REFERENCES

1. Salchi-Sourmaghi M.H., Farsam H., Shafiee A. and Habibinia M., Phytomedicine, **3(1)** (1996) 300.
2. Almas K. and Al-Lafi T.R., MAPA,.**16(2)** (1995) 206.
3. Cragg G.M., Schepartz S.A.,Sufness M. and Grever M.R., J. Nat. Prod. ,**56**(1993)1657.
4. Pantazis P., J. Biomed. Sci., **3**(1996) 14.
5. Sundravelan R., Desireddy B. and Ciddi V., Ind. J. Biol., **3** (2004) 452
6. MurashigeT. and Skoog F.A.,Physiol.Plant,**15**(1962)473.
7. Banerjee S.,Upadhyay N.,Kukreja A.K., Ahuja P. S., Kumar S., Saha G.C.,Sharma R.P. and Chattopadhyay S.K., Planta Medica, **62** (1996) 333.
8. Whitmer S.,Verpoorte R. and Canel C., Plant Cell Tiss. Org. Cul., **53** (1998)135.
9. Komaraiah P., Kavi Kishore P.B. and Ramakrishna S.V., Biotech. Lett., **23** (2001) 1269.
10. Nahalka J., Blanarik P., Gemeiner P., Matusova E. and Partlova I., J. Biotechnol., **49**(1996)153.
11. Akcam-Oluk E.,Demiray H. and Gurel E., Plant Cell. Biotech. Mol. Bio, .**4(1-2)** (2003) 91.
12. Xu J. F., Xie J., Han A. M., Feng P.S. and Su Z.G., J. Chem. Technol. Biotechno., **72** (1998) 227.
13. Paska C., Innocenti G.,Kunavan M.,Laszio M. and Szilagyi L., Phytochemistry, **52** (1999)79.
14. Komaraiah P., Ramakrishna S.V., Reddanna P. and Kavi Kishore P.B., J. Biotechnol., **101** (2003)181
15. Honda Y., Inaoka H., Takei A., Sugimura Y. and Otsuji K., Phytochemistry, **41** (1996)1517.
16. Jackson A.H. ,Naidoo B., Smith A.E., Bailey A.S. and Vandrevala M.H., Commun., (1978) 779.
17. Verpoorte R., J. Nat. Prod, **49** (1986)1
18. Malik S., Ahmad S.S.,Haider S.I. and Muzaffar A. ,Tetrahedron Lett , **28(2)** (1987)163.
19. Rosenberg E., Williamson K.L. and Roberts J.D. , Org. Magn. Reson., **8**(1976)117.
20. Pouchert C.J., The Aldrich library of NMR spectra. Aldrich Chemical Co. Inc.,Milwaukee., **2** (1983)532.

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