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Phytochemical Content of Leaf and *In Vitro* Established Callus Culture of *Pisonia alba* Span

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Abstract: ***Callus culture of Pisonia alba span. were established from leaf explant. Different growth regulators greatly influenced the growth of callus cultures. Leaf derived callus grown on Murashige and Skooge (MS) medium fortified with different concentration (1, 1.5, 2,2.5 ppm) of2, 4- Dichlorophenoxy acetic Acid, Indole - 3-acetic Acid, Indole - 3-butyric Acid Naphthalene Acetic Acid,6- Benzyl Adenine or 6 Benzyl Amino Purine and Kinetin. Accumulation of bioactive compounds namely Diethyl Phthalate, 2-Pentadecanone, 6,10,14-trimethyl, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, Phytol, 9,12-Octadecadienoic acid, ethyl ester, Ethyl Oleate, Stigmasterol and gamma.-Sitosterol from callus culture was detected and confirmed by G C- Mass Spectrum Analysis.***

Keywords: *Pisonia alba*, Callus culture, phytochemicals, Diethyl Phthalate, Ethyl Oleate, Stigmasterol and gamma, Sitosterol.

1. Introduction

Pisonia alba span (*Nyctaginaceae*) is widely distributed throughout India and it is a evergreen commonly grown lettuce tree. Leaves, stem and root of this species are extensively used by the tribal's in the preparation of several folk medicines. It is used as an antidiabetic, anti-inflammatory agent, and used in the treatment of ulcer, dysentery and snake bite. Medicinal plants are a valuable source of a vast array of chemical compounds; they synthesize and accumulate extractable organic substances in quantities sufficient to be economically useful as raw materials for various commercial applications. Industrialization coupled with urbanization is constantly putting pressure on natural resources. Hence, the *in vitro* culture is an imperative issue and action to be taken at the earliest. Plant tissue culture technology holds great promise for micro-propagation, conservation, and enhancement of the natural levels of valuable secondary plant metabolites and to meet pharmaceutical demands for which conventional methods possess limitations, *in vitro* multiplication provides the way out. There are sufficient reports available about protocols on *in vitro* micro-propagation of many medicinal species. In view of its medicinal importance, presence of active compounds the present study reports the prime protocol for regeneration of callus from leaves of *Pisonia alba*.

2. Material and Methods

The explants were surface sterilized following the procedures as described by Janarthanam and Sumathi, (2013). The *Pisonia alba* explants were collected from healthy potted plant from Thiruvannamalai, Tamil Nadu, India. The surface sterilized explants were cultured in Murashige and Skoog (1962) medium. A standard approach of Latin square method (Collin and Edwards, 1998) was followed in screening of PGR by manipulating the concentration of auxins and cytokinins individually and in combinations. A range of 7 concentrations of auxins and cytokinins (0.1mg, 0.25 mg, 0.5 mg, 1.0 mg, 2.5 mg, 5.0 mg, 10 mg/L) were used in this study. The growth measurement of the callus was determined by following the method as described by Rao and Ravishankar, (2000). Growth of the callus was measured from its biomass in terms of fresh (FW g/L) and dry weight (DW g/L). Fresh weight of callus was measured after removing the excess moisture and agar adhering to the callus surface using blotting paper. Dry weight of callus was determined by drying the callus in hot air oven at 60 °C for 24 h and the result was expressed in g/L DW culture. Primary callus was established from the nodal explants. For secondary callus production, a small portion of primary callus was sub-cultured periodically once in three weeks. The secondary callus was used for all experimental studies. The age of callus was determined by following the method as described by Janarthanan et al., 2010. About 0.5gm of actively growing secondary callus culture of *Pisonia alba* was inoculated in 250mL Erlenmeyer flask containing 50 mL of MS solid medium supplemented with 2,4-D 4.52 µM + NAA 2.69 µM and BA 2.22µM. The culture was incubated under 16/8h photoperiod at 25 ± 1°C. Initial weight of the callus biomass was measured in terms of fresh (FW g/L) and dry weight (DW g/L). Observations were made from the 9th day after incubation up to 36th day with three days intervals and callus biomass yield data were recorded.

Gas Chromatography-Mass Spectrometry Analysis

For the Identification of bioactive components, the extract (best fraction) was subjected to GC-MS analysis. GC-MS analysis was carried out on a GC-MS -5975C agilent system comprising an auto sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument, employing the following conditions: column Elite-1 fused silica capillary column (30×0.25 mm ID × 1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1.51 ml/min and an injection volume of 1µl was employed (split ratio of 10:1) injector temperature 240ºC; ion-source temperature 200ºC. The oven temperature was programmed from 70ºC (isothermal for 2 min), with an increase of 10ºC/min, to 300ºC/min, ending with a 9 min isothermal at 300ºC. Mass spectra were taken at 70eV; with a scan range 40-1000 m/z. Solvent cut time was 5 min; MS start time being 5 min; MS end time being 35 min; Ion source temperature set to 200ºC and interface temperature being 240ºC.

Identification of Components

Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the component of the test materials were identified.

G C- Mass Spectrum Analysis*:* Hewlett – Packed 6890 gas chromatograph (Agilent Technologies, CA) Connected to a HP5973 mass selective detector was used. Separations were performed on an Agilent ultra 2 fused silica capillary column (12m length, 0.2 mm internal diameter). Helium was used as the carrier gas at a flow rate of 1ml/ min. Samples were injected in split less mode. Initial column temperature was 100°C and it was increased to 400 ºC. Ions were generated by the electron – ionization mode at 70 Ev. Diluted samples of 1 ppm concentration were injected. Total GC running time was 36 min. Interpretation of mass spectrum was done with reference to National institute of Standard and Technology (NIST) database.

3. Result and Discussion

The callus were cultured with various concentration of Growth hormones (Jitendra Mehta *et al*, 2012). Individual effect of selected auxins and combined effect of auxins and cytokinin on callus culture was studied and the results were tabulated in the Table 1,2 & 3. Among these combinations 2,4 D and NAA shows the production of callus at 4.52(µM) concentration produces Fresh weight of 62.56g/l and dry weight of 5.03g/l. In cytokinin, KN at 4.65(µM) concentration produces Fresh weight of 68.65g/l and dry weight of 5.43g/l. Combined effect of auxins and cytokinin, 2,4 D at 4.52, NAA at 2.69 , BA at 2.22 concentration produces Fresh weight of 143.5g/l and dry weight of 9.03g/l (Minocha *et al*,1987). To produce callus biomass in solid medium at different age were investigated and the result were tabulated in the Table 4 and In 24thDay of culture it produces 143.5g/l fresh weight and 9.03g/l of callus biomass. In the extraction of Active compounds by Gas Chromatography- Mass Spectrum Analysis (GC-MS) were analyzed (Deepaphilip et al, 2011) and relative percentage of the chemical constituents in crude extracts was expressed as percentage by peak area normalization.in Graph:1. The results concerning to GC-MS analysis led to the identification of number of compounds from the GC fractions of the Callus extract of *Pisonia alba*. These compounds were identified through mass spectrum attached with GC (samidurai k etal,2014). The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (%) were tabulated in *Table 5*. In this GC - MS analysis shows the presence of 13 compounds , Diethyl Phthalate(62.37 %), 2-Pentadecanone, 6,10,14-trimethyl(1.09%), n-Hexadecanoic acid(5.00%) , Hexadecanoic acid ethyl ester (4.60%), Phytol (8.30%), 9,12-Octadecadienoic acid , ethyl ester (1.74%) , Ethyl Oleate (1.02%), Stigmasterol (8.22%) and gamma.-Sitosterol(7.67%).

In the conclusions, Tissue culture technology offers an alternative method for the conservation as well as micropropagation of medicinally important plant resources. Presently there is great demand for the use of plant based medicaments in place of synthetic drugs. As a result of non-scientific exploitation, most of the medicinal plant resources are being threatened and are on the verge of extinction. Therefore, application of this technology provides the raw materials required for the isolation of drugs by the pharmaceutical industries without depleting natural plant resources.

The phytochemical content of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate an urgent step for screening of plants for secondary metabolites. The present research work attempts to assess the importance of active compound in the callus extract of *pisonia alba* to improve the health status of people and also to use in nutraceuticals products of commercial importance. The results indicate that the plant material may become an important source of compounds with health protective potential.

4. Acknowledgements

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Table 1: Individual effect of selected auxins on callus culture of *Pisonia alba*

2,4 D			NAA		
Conc. (µM)	FW g/L	DW g/L	Conc. (µM)	FW g/L	DW g/L
0.45	42.13	3.52	0.54	36.64	2.43
1.13	45.43	4.15	1.34	41.13	4.31
2.26	51.87	4.23	2.69	46.72	3.42
4.52	62.56	5.03	5.37	45.35	3.12
11.31	49.21	4.02	13.43	32.12	2.99
22.62	41.25	3.72	26.85	30.29	2.72
45.25	40.97	3.37	53.71	29.33	2.66

Table 2: Individual effect of cytokinins on callus culture of *Pisonia alba*

BA			KN		
Conc. (µM)	FW g/L	DW g/L	Conc. (µM)	FW g/L	DW g/L
0.44	22.34	2.32	0.46	52.64	4.40
1.11	39.50	3.52	1.16	55.85	4.60
2.22	52.07	4.62	2.32	57.59	4.89
4.44	44.92	3.72	4.65	68.62	5.43
11.10	42.08	3.18	11.61	46.66	4.05
22.20	34.00	3.01	23.23	44.24	3.45
44.40	31.60	2.82	46.47	36.43	3.15

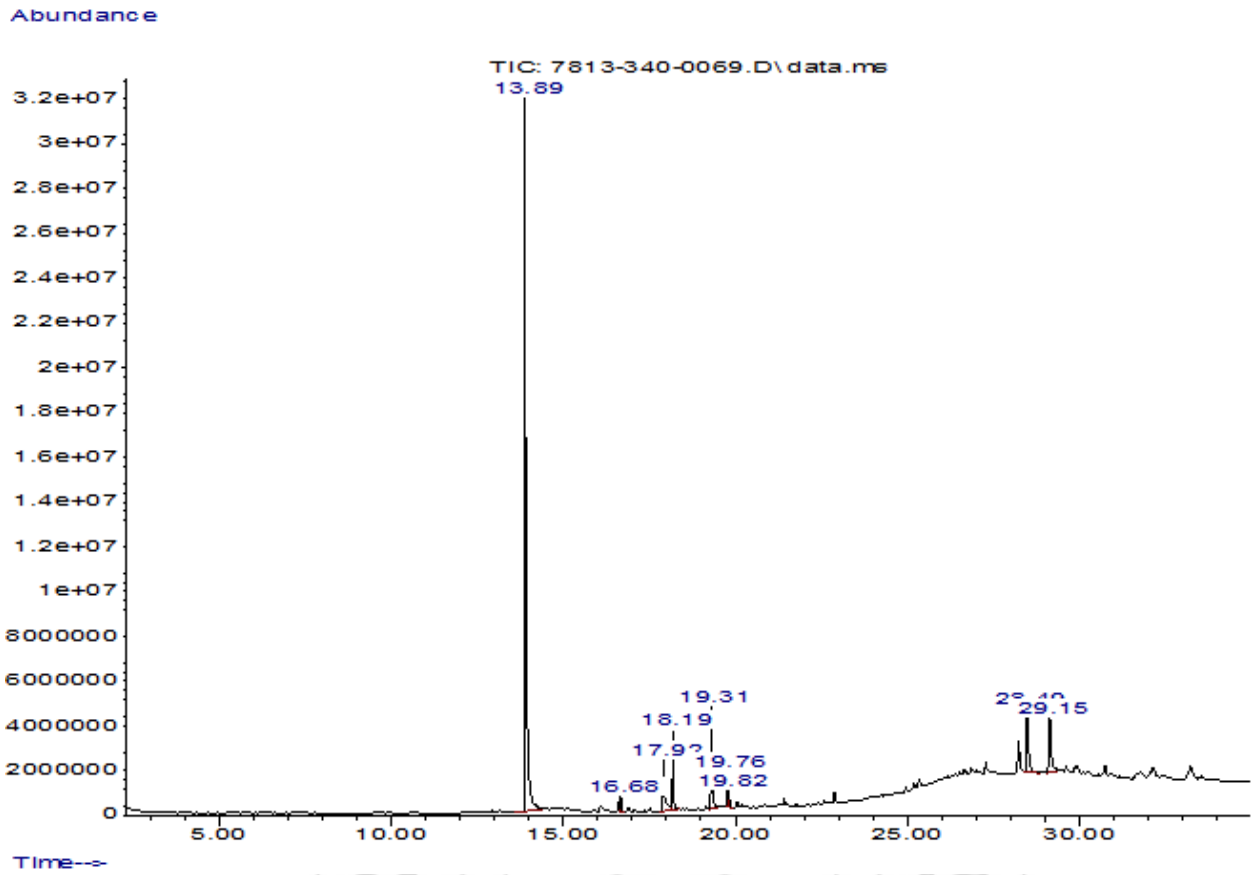
Table 3: Combined effect of auxins and cytokinin on callus culture of *Pisonia alba*

2, 4D	NAA	BA	KN	FW g/l	DW g/l
4.52	-	2.22	-		
-	2.69	2.22	-	93.4	7.02
4.52	-	-	4.65	88.3	7.93

-	2.69	-	4.65	73.3	6.43
4.52	2.69	2.22		143.5	9.03
4.52	2.69		4.65	126.5	6.94

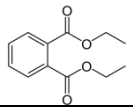
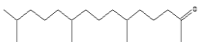
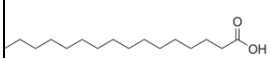
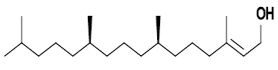
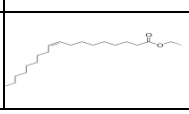
Table 4: Callus biomass in solid medium at different age culture of *Pisonia alba* *

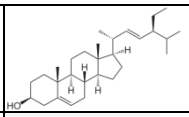
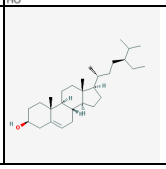
Incubation days	Callus Biomass (Solid medium)	
	FW (g/L)	DW (g/L)
9	40.3	3.02
12	51.7	3.24
15	90.0	6.97
18	121.3	7.85
21	139.0	8.71
24	143.5	9.03
27	141.7	8.93
30	136.3	8.56
33	133.4	8.63
36	132.2	8.12



Graph 1: GC-MS Chromatogram of Aqueous extract of callus of *Pisonia alba*

Table 5: Phytocomponents identified in the callus extract of *Pisonia alba* by GC-MS

No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %	Structure
1	13.891	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.089	62.37	
2	16.679	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	268.27	1.09	
3	17.914	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.24	5.00	
4	18.190	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.27	4.60	
5	19.308	Phytol	$C_{20}H_{40}O$	296.308	8.30	
6	19.758	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308.272	1.74	
7	19.816	Ethyl Oleate	$C_{20}H_{38}O_2$	310.287	1.02	

8	28.486	Stigma sterol	$C_{29}H_{48}O$	412.371	8.22	
9	29.140	gamma.-Sitosterol	$C_{29}H_{50}O$	414.386	7.67	

- Source – Dr.Duke's phytochemical and ethnobotanical Database.

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