IJPSR (2021), Volume 12, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 January 2021; received in revised form, 23 May 2021; accepted, 13 June 2021; published 01 December 2021

EVALUATION OF PHYTOCHEMICAL ANALYSIS OF CALLUS TISSUE FROM INTERNODE EXPLANTS OF TRADITIONAL MEDICINAL PLANTS OF *JATROPHA GOSSYPIIFOLIA* LINN.

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Keywords:

MS medium, BAP, Jatropha, NAA, Antibacterial, GC-MS

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ABSTRACT: *Jatropha gossypiifolia* Linn. (Euphorbiaceae) is an ornamental shrub extensively used in traditional medicine for various ailments. In view of its potential medicinal properties, an attempt has been undertaken to devise an efficient protocol for *in-vitro* propagation from the internodes explants of *J. gossypiifolia* for a rapid and large-scale production as well as to study the phytochemical composition by gas chromatography and mass spectrometry (GC-MS). Further, the petroleum ether and ethyl acetate extracts of callus tissue were subjected to thin-layer chromatography (TLC) as well as for synthesizing silver and gold nanoparticles. The five bands resolved by TLC chromatogram were characterized by Fourier Transform Infrared Spectroscopy (FT-IR) and screened for antimicrobial and antioxidant activities. Internode region of the one-year-old plant was used as explants. Internodal explants are cultured on Murashige and Skoog (MS) medium containing Naphthalene acetic acid (NAA) and 2,4-Dichlorophenoxy acetic acid (2,4-D) both at a concentration of 1.0 mg/L-1 induced the formation of callus between 14-18 days. This combination produced mature cream-colored calli by day 35. Explants growing on MS medium fortified with 2,4-D and benzyl amino purine (BAP), both a concentration of 2.0 mg/L-1 for 35 days, revealed compact light green calli due to greenish pigmentation. Silver and gold nanoparticles as well as the 3rd and 4th bands of TLC by using the ethyl acetate extracts of callus tissue, revealed antibacterial activity. The 3rd and 4th bands of TLC bands obtained by using petroleum ether and ethyl acetate extracts of callus tissue revealed antioxidant activity. A total of 31 compounds were obtained in GC-MS analysis, which belongs to different chemical classes. Among them, phytol, hexadecanoic acid, squalene, 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 9-Octadecenoic acid (Z)-, 2,3-bis(acetyloxy)propyl ester and phthalic acid were some of the major compounds. The presence of these compounds in the callus tissue indicates that they are promising candidates for therapeutic use.

INTRODUCTION: *Jatropha gossypiifolia* Linn. (Euphorbiaceae) is widely distributed all over the tropics and subtropics of Asia, Africa, Central and South America [1]. *J. gossypiifolia* is popularly known as "bellyache bush".

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(12).6403-15

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(12).6403-15

It is so named as it causes bellyache bush poisoning and eventually death in humans and animals, when the poisonous fruits which contain the toxic substance, tox albumin are eaten.

The roots, stem, and leaves of *J. gossypiifolia* have been used to treat various ailments, including intermittent fevers, stomachache, toothache, swollen mammae, eczema, cancer, carbuncles, dysentery, dyspepsia, venereal disease, and for bites of venomous animals [1, 2, 3, 4]. The decoction of the leaf is used for itches, boils, burns, sprains, sores, swelling, eczema, and wound healing [5].

The leaves are also used as blood purifiers [2]. Fresh leaf extract, as well as stem sap, are used to stop bleeding from the nose and to treat skin diseases [6]. Pharmacological studies with different parts of *J. gossypiifolia* are shown to possess antibiotic, antioxidant, anti-inflammatory, antihypertensive, antimicrobial, anticholinesterase, hepatoprotective, insecticidal, pesticide properties, and antidote for poisoning [7, 8, 9].

Anti-inflammatory activity was manifested by the inhibition of acute carrageenan-induced paw edema and cotton pellet-induced granuloma formation in rats administered orally with the ethanolic extract of leaves of *J. gossypiifolia* [10]. Diterpenoids (atrogossones) from *J. gossypiifolia* exhibited antiproliferative activity by inducing G2/M arrest and apoptosis in RKO colon cancer cells [11].

Other diterpenoids like falodone and jatrophone isolated from the roots of *J. gossypiifolia* also revealed antiproliferative activity in A549 (lung cancer cell line) and Hep G2 1886 (colon cancer cell line), respectively [12, 13]. Extracts obtained from different parts of *J. gossypiifolia* and the diterpene jatrophenone have shown antimicrobial activity against various bacterial and fungal strains, including Escherichia coli, *Staphylococcus aureus,* and *Bacillus subtilis*, *Aspergillus fumigatus*, *A. flavus*, *Salmonella typhi*, and *Streptomyces pyogene* [14, 15]. The antioxidant properties of *J. gossypiifolia* extracts were reflected from the significant hydroxyl and superoxide radical scavenging activities and lipoxygenase inhibitory activity [16].

Phytochemical composition of the plant revealed the presence of flavonoids such as apigenin, vitexin, and isovitexin [17], lignins such as gossypifan, gossypilin, gossypidien, jatroiden, jatrodien *etc.* [18], The plant also contains saponins, tannins and phenolic acids [19]. Plant growth regulators play a significant role in callus formation, each having a well-defined effect on callus induction, growth, and development [20, 21, 22]. Cytokinins such as 2,4-Dichlorophenoxy acetic acid (2,4-D), benzyl amino purine (BAP), kinetin and auxins like Naphthalene acetic acid (NAA), Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) have been used for regeneration. Among them, BAP at a concentration of 2.0 mg/L^{-1} was found to be more effective in shoot bud induction. Besides, certain media additives like adenine sulphate, glutamine, and activated charcoal promoted a high frequency of multiple shoot proliferation (90%) within 15-20 days. Regeneration of hypocotyls, petiole, and leaf explants was observed when cytokinins like zeatin, kinetin, and N6 benzyladenine either singly or in combination with IBA were used [23].

In spite of the vast medicinal potentialities of J. *gossypiifolia*, efforts have not been taken to conserve this species from genetic extinction due to overexploitation. This continuous exploitation necessitates to employ *in-vitro* regeneration to produce this species for extraction of secondary metabolites for commercial purposes. Further, even though a number of reports are available to delineate *in-vitro* propagation of callus from other Jatropha species, *J. gossypiifolia* has been scarcely studied for callus formation. Therefore, an attempt has been made to provide a rapid regeneration protocol of this potent medicinal plant from internodal cuttings. Studies were also undertaken to evaluate the phytochemicals present in the callus tissue by gas chromatography and mass spectrometry (GC-MS) and thin layer chromatography (TLC) and examine the anti-oxidant and antibacterial activities of gold and silver nanoparticles prepared using the callus of *J. gossypiifolia*.

MATERIALS AND METHODS:

Source and Sterilization of *J. gossypiifolia* Explants: *Jatropha gossypiifolia* plants grown in the Kalasalingam University Campus were used as a source of explants. The young internodal region of 3rd and 4th node from the apex were collected from one-year-old plants. The explants were washed under running water and then with a soap solution. The explants were surface sterilized with mercuric chloride (0.1%) for 5 min, and rinsed with double distilled water five times.

Culture Conditions of *J. gossypiifolia* Explants: The present study is focused on examining the influence of BAP and NAA on callus induction and growth of *J. gossypiifolia* explants. Tissue culture media were prepared according to the method of Murashige and Skoog (1962). The media contained sucrose (3%) and agar (0.8%) as solidifying agents. Internodes were excised aseptically into small

pieces of 1 × 1 cm and inoculated on 24 basal medium for callus initiation. After a week, these explants were transferred to media containing phytohormones such as BAP (2 mg L^{-1}) and NAA (1 mg L-1), 2,4-D (2 mg L^{-1}) for regeneration. The pH of the media was set 5.6 ± 0.1. Media was steam-sterilized in an autoclave under 15 psi at 121 ºC for 20 min. Growth conditions were maintained at 27 °C with 16/8 h light-dark photoperiod with lux intensity of 3000 by cool white fluorescent tubes (Philips, India).

Preparation of Callus Tissue Extracts: Thirty-five-day-old mature callus tissue was extracted with petroleum ether and ethyl acetate for 8 h and concentrated using a rotary evaporator, and stored in a desiccator until use.

Synthesis of silver and Gold Nanoparticles using Callus Tissue: Silver and gold nanoparticles were synthesized by mixing 10 ml of callus tissue extract with 100 ml of 1 mM aqueous solution of silver and gold nitrates separately with constant stirring at room temperature.

The mixtures were heated at 60 °C and stored in the dark for 24 h. The change of colour of the solution was inspected visually. The reduction of Ag+ to Ag0 was confirmed by the colour change of solution from colourless to brown.

Characterization of Silver and Gold Nano-particles: Silver and gold nanoparticles were characterized by SEM analysis. In order to carry out SEM analysis, the solution containing silver nanoparticles was centrifuged at 10,000 rpm for 20 min and a drop of the sample was loaded on a stub and SEM analysis was then performed using the Scanning Electron Microscope (JSM-6360, JEOL). Compositional analysis and the confirmation of the presence of elemental silver was carried out through Energy-dispersive X-ray Spectroscopy (EDS) using the SEM equipped with an EDX attachment (Carl Zeiss, Germany).

TLC Method: TLC is an analytical technique used to separate a mixture of crude substances into its individual compounds. In the present study, an aliquot of petroleum ether and ethyl acetate extracts were spotted on TLC silica gel plates (10 × 15 cm) separately. The plates were developed using hexane and ethyl acetate (8:2) as the mobile phase. After the run was over, the plates were visualized under visible and UV light (240 and 300 nm). In the present study, the chromatogram revealed five distinct bands. The separated bands were marked and their retention factor (Rf) values were calculated and recorded. The chromatogram was then photographed.

The five bands obtained using petroleum ether and ethyl acetate extracts were scratched off separately, dissolved in alcohol, filtered, concentrated, and then used for the antioxidant assay. For antibacterial assay, the TLC products obtained from ethyl acetate extract alone were used.

FTIR Analysis: FTIR spectral analysis was performed by TLC bioautographic method using the recovered concentrates of five TLC bands obtained from the ethyl acetate extracts of 35-day old callus tissue. FTIR spectral analysis was performed in FTIR instrument (IRTRACER-100, Shimadzu, Japan) with PC-based software and data processing. As mentioned earlier, each band of TLC was removed separately, mixed with absolute ethanol, filtered, and concentrated. The recovered concentrates of each band were mixed with KBr (100 mg) and made into pellets by applying pressure.

Antibacterial Activity: Antibacterial activity was screened by two ways *viz.,* TLC bioautographic method as well as by silver and gold nanoparticles obtained from the ethyl acetate extract of 35-day old callus tissue. All bacterial strains *Staphylococcus aureus* (MTCC 96) (Gram-positive bacteria) *Escherichia coli* (MTCC 1652), and *Pseudomonas aeruginosa* (MTCC 2453) (Gram-negative bacteria) were provided by the Microbiology laboratory of the Meenakshi Mission Hospital. All bacterial strains were subcultured on nutrient agar for 24 h favorable to their growth prior to testing.

In the present study, the TLC chromatogram obtained by using ethyl acetate fraction of callus tissue showed five bands. Each of the five bands of TLC was scratched off separately, mixed with ethanol, filtered, and concentrated. The recovered concentrates of each band were then tested for antibacterial activity by the agar well diffusion method. at 37 °C.

Muller Hinton agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petriplates and then solidified. Wells of 7 mm diameter were made on the agar with cork borer. Silver and gold nanoparticles (20 µl) prepared using ethyl acetate callus extract were added into wells made in agar plates and allowed to diffuse for 5 min. DMSO alone added to the wells, served as test control, while wells loaded with ampicillin (20 µg/ml) served as a positive control. The plates were kept inverted in an incubator set at 37 ºC for 24 h. The growth inhibitory zones around the wells were measured using a ruler in centimeters and taken as an indication of antimicrobial activity. The assays were carried out in triplicates.

Determination of Total Antioxidant Activity: Antioxidant activity was evaluated by TLC bioautographic method. Each of the five bands of TLC chromatogram obtained by using petroleum ether and ethyl acetate extract was removed separately, mixed with absolute ethanol, filtered, and concentrated. The recovered solutions of each band was then tested for total antioxidant activity

The total antioxidant assay is based on the reduction of molybdenum (VI) to green phosphate/molybdenum (V) complex at an acidic pH by the sample analyte [25]. An aliquot from the recovered solutions (0.3 ml) of each TLC band was added to 1 ml of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate, heated to 95 °C for 90 min and then cooled to room temperature. The intensity of the green colour developed was read at 695 nm against a reagent blank using a double beam spectrophotometer (UV-160 A, Shimadzu Corporation, Kyoto, Japan). The total antioxidant activity is expressed as g equivalent of ascorbic acid.

Phytochemical Screening of Callus Tissue by GC-MS: The phytochemical analysis in the ethyl acetate extract of 35 day old callus tissue was carried out on GC-MS model HP 6890 (Agilent Technologies Ltd) fitted with an HP-5 MS (5% phenylmethyl siloxane) capillary column 30 m × 250 µm × 0.25 µm. The sample (1 µl) was injected to the injected port. The injector temperature was adjusted at 250 °C, while the detector temperature was fixed to 280 °C. The column temperature was raised from 40 °C to 220 ºC at a rate of 6 ºC/min. The flow rate of the carrier gas, helium was set 1 ml/min, and the split ratio was 1:50. The sample was vaporized, and the various components in the sample were separated and analyzed. The Mass spectra were acquired in scan mode (70 eV) in the range of 50–550 m/z. The spectral peak produced by each component was recorded. The retention time of the components was determined by matching the spectra with that of reference compounds.

Identification of Components: In the MS Program, National Institute Standard and Technology (NIST), Version 14.0, Wiley 8.0 library database of NIST having more than 62000 patterns were used for identifying the chemical components. The unknown phytochemicals were identified by comparing the spectra of both known and unknown compounds stored in the NIST database.

Statistical Analysis: The data were analyzed by one-way analysis of variance (ANOVA) using Statistical Software. The measurements are expressed as mean ± standard errors of the mean (SEM). A significance level of 0.05 was used for all statistical tests.

RESULTS AND DISCUSSION:

Callus Formation and Growth: The explants selected from mature plant internodes of *J. gossypiifolia* were cultured in callus induction medium at 26°C in the dark for 55 days. The callus formation was induced by a combination of cytokinin and auxin, which act synergistically to promote cell division, growth, development, differentiation, and the formation of plant organs [20, 22]. The auxin 2, 4-D plays a key role in the induction of callus [26]. Later, it was found that the addition of cytokinins acts in concert with auxins to enhance callus regeneration [27]. MS media supplemented with only NAA has no significant effect on shoot proliferation [21]. However, a number of studies have shown that NAA was the best plant growth hormone, and the optimal concentration was 1 mg/L^{-1} for producing green, compact, and fast-growing Jatropha callus (Rajore and Batra, 2007). In the present investigation, callus induction was achieved when explants were inoculated on MS media fortified with 2,4-D and NAA. Callusing

was initiated between 14 and 18 days of explants transformation **Fig. 1A-C**. This is consistent with earlier studies of Rajore and Batra (2007), who have shown the induction of callus between 16-20 days from shoot tip explants of *J. curcus*. The callus growth reached 100% by 35 days **Fig. 1**.

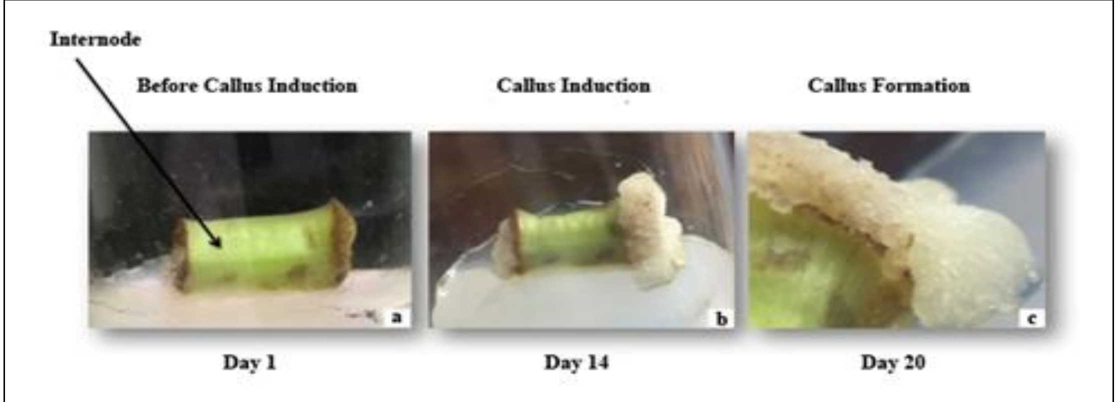

FIG. 1: DIFFERENT STAGES OF CALLUS DEVELOPMENT ON MS MEDIA SUPPLEMENTED WITH NAA (1 MG/L^{-1}) AND 2,4-D (1 MG/L^{-1})

Thirty-five days old explants growing on media containing 2,4-D (2 mg/L^{-1}) with NAA (1 mg/L^{-1}) produced white, cream-coloured calli. On the other hand, explants growing on MS medium fortified with 2,4-D (2 mg/L^{-1}) and BAP (2 mg/L^{-1}) for 35 days revealed compact and light green calli due to greenish pigmentation **Fig. 2A** and **B**. This finding gets further support from the study of de Oliveira et al. (2017), who had shown increased frequency of compact and light green calli, when MS media contained BAP (2 mg L^{-1}). The grren calli have features opposite to that of cream-colored and friable ones, which have lower chances to differentiate. These observations infer that BAP plays a significant role in the induction and growth of green callus of *J. gossypifolia*, which may also imply BAP has an effect to preserve greenish pigmentation as well as to prevent senescence in callus tissue.

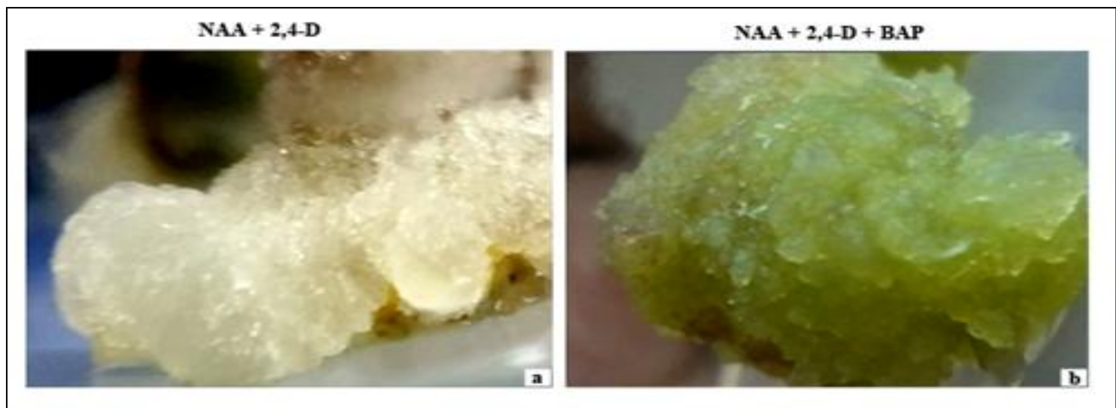

FIG. 2: CALLUS DEVELOPMENT ON MS MEDIA SUPPLEMENTED WITH NAA (1 MG/L^{-1}) + 2,4-D (1 MG/L^{-1}) WITH AND WITHOUT BAP (MG/L^{-1})

Characterization of Silver and Gold Nanoparticles: Silver and gold nanoparticles were purified and scanned by SEM analysis Fig**. 3, 4,** and **5**. SEM is the most widely used technique for characterizing nanoparticles in terms of the physical morphology of the particles. In the present study, SEM images suggest that biosynthesized silver nanoparticles are almost spherical in structure **Fig. 4A-C**.

The EDS profiles of the nanoparticles confirmed the presence of a characteristic silver signal at approximately 3 keV **Fig 4C**, which is typical for the absorption of silver nanoparticles due to surface plasmon resonance confirming that silver nanoparticles were biosynthesized successfully using *J. gossififolia*. Similarly, the presence of gold elements in the EDX analysis confirmed the formation of gold nanoparticles **Fig 5A-C**.

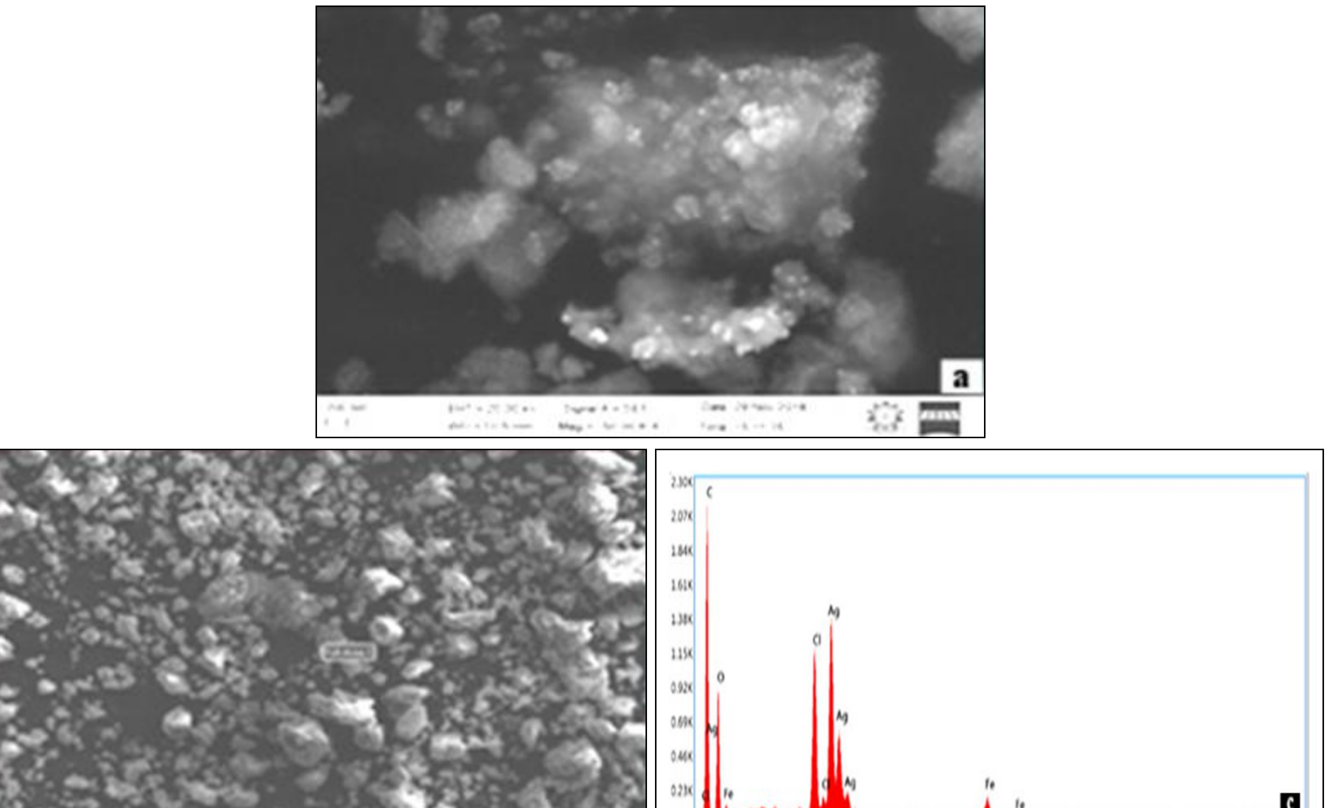

FIG. 3: SEM IMAGES WITH EDX ANALYSIS OF SILVER NANOPARTICLES

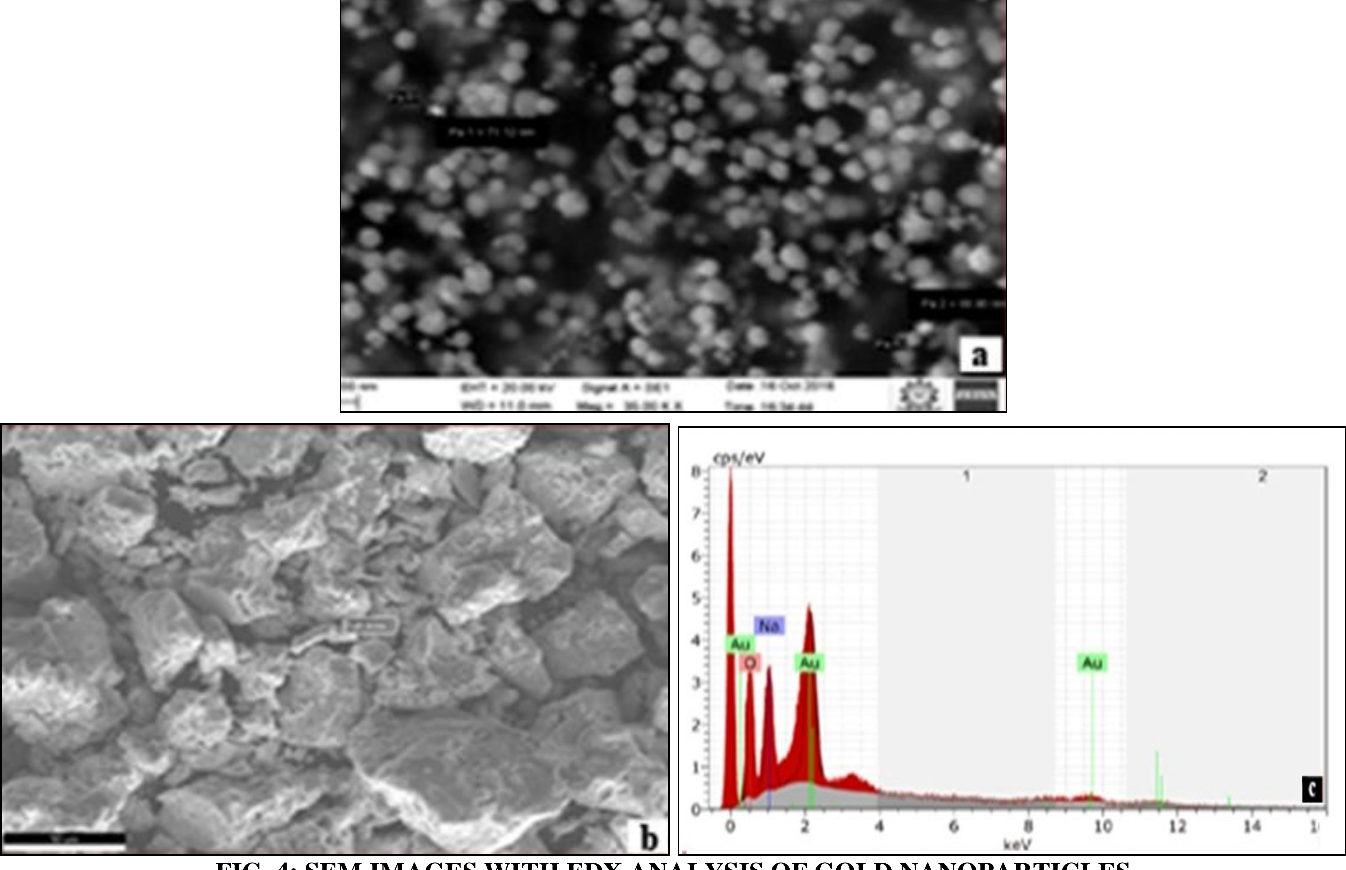

FIG. 4: SEM IMAGES WITH EDX ANALYSIS OF GOLD NANOPARTICLES

Legend for Fig. 4: Scanning electron microscopy images (a and b) represents gold nanoparticles synthesised using ethyl acetate extract of callus of *J. gossypiifolia*. **Fig. 4C** represents the Energy-Dispersive X-ray microanalysis of gold nanoparticles.

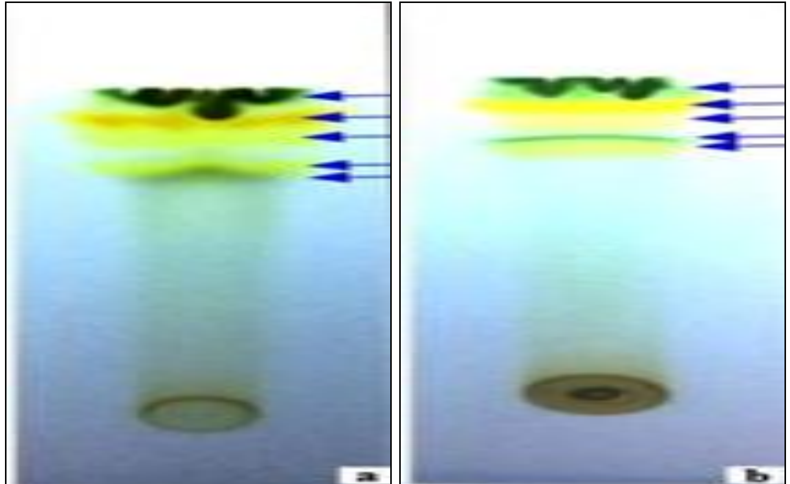

FIG. 5: TLC CHROMATOGRAM SHOWING BANDS A – PETROLEUM ETHER EXTRACT; B – ETHYL ACETATE EXTRACT

Antibacterial Assays of Silver and Gold Nanoparticles and TLC Chromatogram Products: Silver and gold nanoparticles obtained by using 35-day old callus tissue were tested for antibacterial activity. The gold nanoparticles showed the highest antimicrobial activity with an inhibition zone of 2.1 cm against Gram-negative *P. aeroginosa* compared to silver nanoparticles, which showed an inhibition zone of 1.0 cm. Gold nanoparticles also inhibited gram-positive *S. aureus* with inhibition zone of 1.9 cm, while silver nanoparticles showed no antibacterial activity **Fig. 6**.

FIG. 6: ANTIBACTERIAL ACTIVITY OF SILVER AND GOLD NANOPARTICLES FROM CALLUS TISSUE AGAINST GRAM-NEGATIVE *P. AEROGINOASA* AND GRAM-POSITIVE *S. AUREAS*

Antibacterial activity was also evaluated by TLC bioautographic method. The recovered concentrates of each of the five TLC bands obtained by using ethyl acetate extract of 35-day old callus tissue **Fig. 7A**. were tested against *P. aeroginosa* (Gram-negative) and *S. aureus* (Gram-positive bacteria) by well diffusion method. Of the five bands of TLC tested, both 3rd and 4th bands alone have shown pronounced antibacterial activity against the test pathogens with zones of inhibition varying between 0.9-1.0 cm against Gram-positive bacteria *S. aureus*. In Gram negative bacteria *P. aeroginosa*, 3rd and 4th bands of TLC inhibited bacterial growth with zones of inhibition 1.2 and 1.7 cm, respectively **Fig. 7B** and **C**, **Table 1**.

The positive effects observed with the concentrates of TLC bands-3 and 4 indicate that the *J. gossypiifolia* callus synthesizes compounds responsible for antibacterial activity. This is consistent with a number of earlier studies, which have shown good antibacterial activity in the leaf, shoot and root extracts of *J. gossypiifolia* [28, 29, 15].

FIG. 7: ANTIBACTERIAL ACTIVITY AGAINST (A) GRAM-NEGATIVE *P. AEROGINOSA* AND GRAM-POSITIVE (B) *S. AUREAS* BY TLC BIOACTOGRAPHIC METHOD

TABLE 1: ANTIBACTERIAL ACTIVITY OF RECOVERED CONCENTRATES OF TLC BANDS AND SILVER AND GOLD NANOPARTICLES FROM CALLUS TISSUE OF *J. GOSSYPIIFOLIA*

S. no.	Name of the micro-organism	TLC bands In cm							Nanoparticles In cm			
		+ve control (Ampicillin 20 µg/ disc)	Control	TLC band 1	TLC band 2	TLC band 3	TLC band 4	TLC band 5	+ve control (Ampicillin 20 µg/ disc)	Control	Silver nanoparticls	Gold nanoparticls
1	*P. aeroginosa* (MTCC 2453	1.6	-	-	-	1.2	1.7	-	1.2	-	-	1.9
2	*S. aureus* (MTCC 96)	1.4	-	-	-	1.0	0.9		1.1	-	1	2.1

Antioxidant Activity: The total antioxidant activity of recovered concentrates of five TLC bands obtained using petroleum ether and ethyl acetate extracts of 35-day old callus tissue were evaluated by comparing with that of ascorbic acid. Of the 5 TLC compounds tested, 3rd and 4th bands obtained using both petroleum ether and ethyl acetate extracts expressed the highest antioxidant activity, which has surpassed the reference compound, ascorbic acid **Fig. 8**. This finding gets support from a number of earlier studies, which have shown significant hydroxyl and superoxide radical scavenging activities of aqueous leaf crude extract of *J. gossypiifolia* [16, 4]. These authors have claimed that the high content of phenols, tannins, and flavonoids present in the leaves are responsible for the antioxidant activity of this plant.

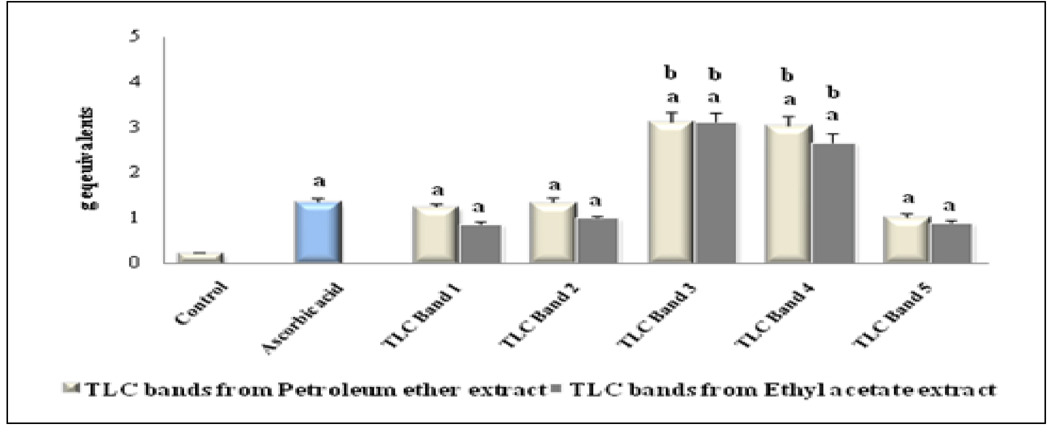

FIG. 8: ANTIOXIDANT ACTIVITY BY TLC BIOAUTOGRAPHIC METHOD USING ETHYL ACETATE EXTRACT OF CALLUS TISSUE OF *J. GOSSYPIIFOLIA*

FTIR Analysis: In the present study, FTIR spectra were obtained **Fig. 9** from the recovered concentrates of five TLC bands resolved by using ethyl acetate extracts of 35-day old callus tissue. The 5 bands obtained on the TLC using ethyl acetate extract of *J. gossypiifolia* callus tissue are presented in **Fig. 3B**. The five bands of TLC were chaacterixed by FTIR in the spectral region of 500 to 4000 cm^{-1}. A characteristic absorption maxima at 1096 cm^{-1}, indicating the presence of C-O) for a hydroxyl (-OH), which was observed in TLC bands -2, -3 and -5 except band-3. Apart from this characteristic band, the five TLC bands showed the absorption maxima at 3000-4000 cm^{-1}, which corresponds the hydroxyl groups. The absorption maxima at 2882 cm^{-1} and 2380 cm^{-1} (for C-H stretching), at 1641 cm^{-1} (C=C stretching), 1553-1565 cm^{-1} (C=O aromatic stretch), 974 cm^{-1} (C-H bending of aromatic hydrocarbons) and 798 cm^{-1} (aromatic carbons) were also observed **Table 2**.

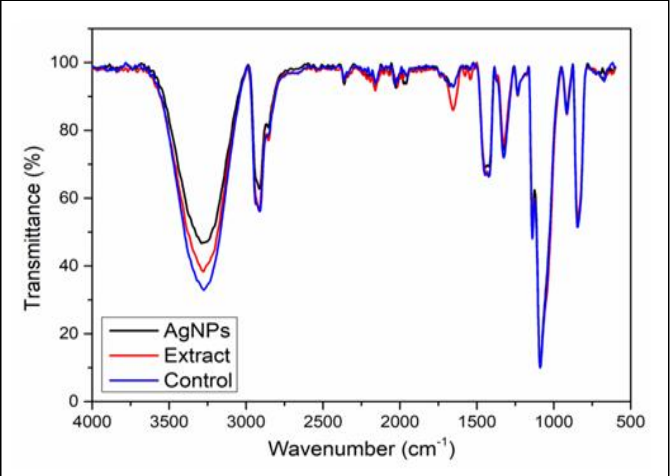

FIG. 9: CHARACTERISATION OF FIVE TLC BANDS BY FTIR

TABLE 2: SHOWS THE CHARACTERISATION OF FIVE TLC BANDS BY FTIR AND FUNCTIONAL GROUPS

TLC band 1	TLC band 2	TLC band 3	TLC band 4	TLC band 5	Functional groups	
Wave numbers cm^{-1}						
3466.08	3485.01	3458.37	3462.22	3450.65	O-H	Hydrogen bonded alcohols, phenols
2881.65	2881.61	2881.65	2881.65	2879.72	=C-H	Alkanes
2387.87	2361.23	2349.30	--	2367.01	C-H Stretching	Alkanes
--	--	1869.02	--	--	Unknown	
1639.49	1638.48	1637.56	1635.64	--	C=C stretch	Alkene
1552.70	--	1554.63	--	1552.70	C=O aromatic streching	Alkene
--	1083.64	1095.57	--	1095.57	C-O	Ester
974.06	974.05	974.05	--	--	C-H bending	Alkane
798.53	798.53	798.53	798.53	798.53	Aromaatic carbons	
468.70	488.71	466.77	466.56	466.77	S-S	Aryl disulphides

Phytochemical Analysis of Callus Tissue Using GC-MS: Phytochemical screening of callus is required to identify the nature of bioactive components in order to discover novel therapeutic agents with better efficacy. In the present study,

The chromatogram peaks were identified by comparing with the spectra of known and unknown components stored in the NIST library.

GC-MS profiling of ethyl acetate extract of *J. gossypiifolia* callus revealed the presence of thirty-one phytocomponents. A distinct chromatogram of *J. gossypiifolia* callus is shown in **Fig. 10**.

The bioactive compounds with their retention time (RT), molecular formula, molecular weight, peak height and area are represented in **Table 3**. The phytocomponents in the callus of *J. gossypiifolia* showed a chromatogram with a retention time ranging from 3.94 to 23.61.

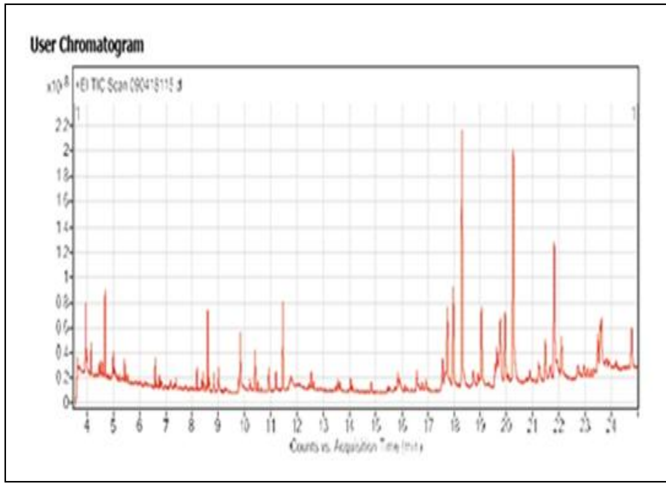

FIG. 10: GC-MS CHROMATOGRAM OF ETHYL ACETATE EXTRACT OF CALLUS TISSUE OF *J. GOSSYPIIFOLIA*

TABLE 3: COMPOUNDS IDENTIFIED BY GC-MS ANALYSIS FROM THE ETHYL ACETATE EXTRACT OF *J. GOSSYPIIFOLIA*

Peak	RT	Name of the compound (IUPAC Names)	Molecular formula	Mol. wt	Structure of the compound
1	3.94	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	134.1	
2	4.16	Succinimide	$C_4H_5NO_2$	99	
3	4.68	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	134.1	
4	4.77	Propane, 2-fluoro-2-methyl-	C_4H_9F	76.1	
5	5.55	Ethyl Acetate	$C_4H_8O_2$	88.1	
6	8.19	1-Undecene, 8-methyl-	$C_{12}H_{24}$	168.2	
7	8.60	1,2-Bis(2-cyano-2-propyl)- hydrazine	$C_8H_{14}N_4$	166.1	
8	8.60	Cyclohexanol, 5-methyl-2-(1- methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-)-	$C_{10}H_{20}O$	156.2	
9	8.83	Phthalic acid, nonyl tridec-2- yn-1-yl ester	$C_{30}H_{46}O_4$	470.3	
10	9.01	3,7,11,15-Tetramethyl-2- hexadecen-1-ol	$C_{20}H_{40}O$	296.3	
11	9.84	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.2	
12	10.40	1-(2,3-Dimethylphenyl)ethanone	$C_{10}H_{12}O$	148.1	
13	11.46	Phytol	$C_{20}H_{40}O$	296.3	
14	11.76	Pentane, 3-ethyl-	C_7H_{16}	100.1	
15	11.77	(E)-Hex-3-enyl isobutyl carbonate	$C_{11}H_{20}O_3$	200.1	
16	15.85	Nonane, 1-iodo-	$C_9H_{19}I$	254.1	

17	16.58	16-Hexadecanoyl hydrazide	$C_{16}H_{34}N_2O$	270.3	
18	17.55	1,2-Diheptylcyclopropene	$C_{17}H_{32}$	236.3	
19	17.74	1-Hexanethiol, 2-(9- borabicyclo[3.3.1]non-9- yloxy)-	$C_{14}H_{27}BOS$	254.2	
20	17.96	3,5-Dimethyldodecane	$C_{14}H_{30}$	198.2	
21	18.30	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-	$C_{25}H_{40}O_6$	436.3	
22	19.04	Squalene	$C_{30}H_{50}$	410.4	
23	19.76	1,13-Tridecanediol, diacetate	$C_{17}H_{32}O_4$	300.2	
24	19.92	2-Butyl-1-ethyl-1,2- azaborolidine	$C_9H_{20}BN$	153.2	
25	19.94	threo-2,5-Dimethyl-2-(2- methyl-2-tetrahydrofuryl)tetrahydrofuran	$C_{11}H_{20}O_2$	184.1	
26	20.25	9-Octadecenoic acid (Z)-, 2,3-bis(acetyloxy)propyl ester	$C_{25}H_{44}O_6$	440.3	
27	21.81	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	242.3	
28	21.82	6,7-Dimethyl-9-oxo-1,5-diazabicyclo[5.2.0]non-5-ene	$C_9H_{14}N_2O$	166.1	
29	22.09	Cyclopentane, 1,1'- hexadecylidenebis-	$C_{26}H_{50}$	362.4	
30	23.47	gamma-Sitosterol	$C_{29}H_{50}O$	414.4	
31	23.57	Sulfurous acid, 2-ethylhexyl hexyl ester	$C_{14}H_{30}O_3S$	278.2	
32	23.61	1-Cyclohexyl-1-(4- methylcyclohexyl)ethane	$C_{15}H_{28}$	208.2	

Among the identified [31] phytocomponents, some of them possess promising bioactivities. Phytol is a diterpene that possesses antimicrobial, antioxidant, anticancer, anti-inflammatory, anti-diuretic, immune-stimulatory, and anti-diabetic activities [30, 31]. Hexadecanoic acid is known to exhibit strong antimicrobial and anti-inflammatory, antioxidant and cancer preventive activities. It is useful for the management of eye infections as well as bruises and erupted skins [32, 33, 34, 35]. Squalene is a triterpene that act as natural antioxidants and possesses various pharmacological activities like antibacterial, antioxidant, anti-tumor, cancer preventive, chemopreventive and diuretic properties.

It is also a immunostimulant and a lipoxygenase-inhibitor 36, 37. 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z, Z, Z)- is a fatty acid ester, which possesses anti-inflammatory, cancer preventive, antihistaminic, antiarthritic, anticoronary, antieczemic, antiacne, hepatoprotective, antiandrogenic and hypocholesterolemic properties 38, 39. 9-Octadecenoic acid (Z)-, 2,3-bis(acetyloxy)propyl ester is also a fatty acid ester, which possesses antifungal. anti-bacterial, anti-inflammatory, anti-alopecic, anemia-genic, antitumour, choleretic, dermatitigenic, antiandrogenic, allergenic, and hypocholesterolemic properties [34]. It is also a immunostimulant as well as lipoxygenase and 5α reductase inhibitor. 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol is used in the treatment of asthma, Further, it is cancer-preventive and possesses anti-inflammatory and antimicrobial activities [40, 41]. Based on the spectral data, it was found that the callus of *J. gossypiifolia* contained a large number of bioactive compounds. The analysis separated and identified various phytochemicals belonging to different chemical classes. The presence of various bioactive compounds justifies the propagation and use of this callus tissue for phytopharmaceutical purposes.

CONCLUSION: Taken together, the present study provided a rapid protocol for callus initiation and growth derived from the internode region of year old plant. The petroleum ether and ethyl acetate extracts of thirty-five-day-old callus tissue were resolved into 5 bands by TLC. Of the five bands, the third and fourth bands of TLC revealed antimicrobial and antioxidant activities. GC-MS analysis revealed 31 compounds of different classes. The presence of various bioactive compounds justifies it uses for various ailments by traditional practitioners.

ACKNOWLEDGEMENT: We thank the Vice President, Chancellor, and Vice-Chancellor, Kalasalingam University, Krishnankoil, Viruthunagar District, Srivilliputhur - 626126 Tamil Nadu, India, for their constant encouragement to carry out this work.

CONFLICTS OF INTEREST: The authors do not have any conflicts of interest.

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How to cite this article:
Kalaiyarasan A and Hariram N: Evaluation of phytochemical analysis of callus tissue from internode explants of traditional medicinal plants of *Jatropha gossypiifolia* Linn. Int J Pharm Sci & Res 2021; 12(12): 6403-15. doi: 10.13040/IJPSR.0975-8232.12(12).6403-15.

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