IJPSR (2018), Volume 9, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 17 May, 2017; received in revised form, 17 July, 2017; accepted, 25 July, 2017; published 01 February, 2018

GC-MS ANALYSIS OF CALLUS AND LEAF EXTRACTS OF *IN VITRO* PROPAGATED PLANTS OF *JUSTICIA WYNAADENSIS* (NEES) T. ANDERSON

C. D. Vandana*, K. N. Shanti and S. L. Shantha

Department of Biotechnology, PES Institute of Technology, Bangalore - 560085, Karnataka, India.

Keywords:

GC-MS, *In vitro*, Callus, Micropropagation, *Justicia wynaadensis*

Correspondence to Author:
C. D. Vandana
Research Scholar,
c/o Professor Dr. K.N. Shanti
Department of Biotechnology,
PES Institute of Technology, 100 feet ring road, Banashankari 3rd stage,
Bangalore-560 085, Karnataka, India.

E-mail: vandana.devaiah@gmail.com

ABSTRACT: The present study was aimed at induction of callus, micropropagation of *Justicia wynaadensis* (Nees) T. Anderson *in vitro* by using different explants and study of the phytocomponents present in the aqueous and methanol extracts of callus and leaf of *in vitro* propagated plants through GC-MS analysis. Callus was induced using MS basal medium supplemented with combinations of 2, 4-D, IAA and NAA growth hormones in various concentrations. For micropropagation hormone free MS basal medium and MS basal medium with BAP in various concentrations was used. Callus were obtained on MS medium supplemented with 2mg/L 2, 4-D. *In vitro* shoots were initiated on hormone free MS basal medium and well developed multiple shoots were regenerated on MS basal medium containing 1mg/L BAP. Four different extracts were prepared from callus and leaf of *in vitro* obtained plants and their phytochemical composition were analyzed by GC-MS. The extracts revealed the presence of various phytocomponents, with therapeutically important properties.

INTRODUCTION: Medicinal plants since ages have been used in Indian folklore medicine [1]. Research on natural products is often based on ethnobotanical information [2]. Secondary metabolites from plants are rich in phytochemicals with medicinally useful biological activities, which are used in the production of pharmaceuticals. Application of *in vitro* propagation techniques help in rapid multiplication of rare and economically important plant species [3]. Mass propagation of medicinal plants by application of plant bio-technology has the potential to meet the demand of raw materials used in herbal preparations in pharmaceutical industries. *Justicia wynaadensis* a herb, belonging to the family Acanthaceae, which is endemic to the regions of Western Ghats of South India.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(2).535-43

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(2).535-43

Traditionally native people of Kodagu District, believe that this plant posses medicinal properties and consume during monsoon season.

Studies have reported that extract of *Justicia wynaadensis* lowers cellular cholesterol and cholesteryl ester concentration, and novel inhibitory effect on the uptake of ox-LDL by human macrophage cell line [4]. It was used externally for the treatment of rheumatic swellings by Kurichiar tribes, in Kerala [5]. Reported [6] the anti-inflammatory activity of this plant.

The present study is focused on induction of callus and to identify the phytochemicals present in the aqueous and methanol extracts of callus and *in vitro* regenerated plants of *Justicia wynaadensis* by GC-MS analysis. These *in vitro* regenerated plants may possess therapeutically important phyto compounds which can be used in treatment of diseases.

MATERIALS AND METHODS:

Plant Collection and Explant Preparation: Plants were collected from Kodagu District of

Karnataka, India and authenticated by Dr. K. P. Sreenath, Department of Botany, Bangalore University, Karnataka and it was identified as *Justicia wynaadensis* (Nees) T. Anderson. Stem cuttings with inter nodes were collected and were brought to the research centre to carry out further research work. They were planted in polythene covers with soil, periodically watered for better growth, maintained in shade condition and used as mother plant for further tissue culture work.

In the present study nodal buds, stem, leaves, meristems of *J. wynaadensis* were used as explants for callus induction and micropropagation of plants. Explants were first washed with tap water and surface sterilized by rinsing with 2 to 3 drops of Tween 20 for 15 min. They were treated with Bavestin for 20 minutes, followed by washing in sterilized double distilled water thoroughly. Explants were then treated with 70% ethanol for 30 seconds. Ethanol was removed and the explants were treated with 0.1% Mercuric Chloride solution. After Mercuric Chloride treatment, explants were rinsed with sterilized double distilled water 4 - 5 times for different time periods to remove the traces of Mercuric Chloride. Surface sterilized explants were incised and aseptically placed on the media and incubated under controlled temperature, light and humidity depending on the explant.

Induction of Callus: For callus induction stem and leaves explants were used. MS basal medium was supplemented with (2, 4-D, IAA and NAA) growth hormones individually or in different combination and concentration. Cultures were maintained at 24 ± 2 °C temperature under dark condition and 70-80% humidity.

Micropropagation: For initiation, meristem and nodal buds were used as explants and placed on hormone free MS basal medium. Then to obtain multiple shoots MS basal medium supplemented with growth hormone BAP in various concentrations was used to get true-to-type plants. Cultures were maintained at 24 ± 2 °C temperatures with 16 hrs photo period and 70-80% humidity.

Abbreviations: 6-benzylaminopurine: BAP; 2, 4-dichlorophenoxyacetic acid: 2, 4-D; Indole-3-acetic acid: IAA; Naphthaleneacetic acid: NAA; Murashige and Skoog: MS.

Preparation of Extracts:

Extraction of phytochemicals from Callus and Leaf of Micropropagated Plants: 2gm of stem derived callus was macerated with 20 ml of sterile distilled water and 20ml of methanol separately for 24hrs. Both the extracts were kept in rotary shaker for 20hrs and kept unshaken for 4hr. Leaves of *in vitro* grown plants were excised, dried and ground into fine powder. 1gm of above powder was macerated with 20 ml of sterile distilled water and 20ml of methanol separately for 24hrs. Both the extracts were kept in rotary shaker for 20hr and kept unshaken for 4hr. All the four extracts were filtered using filter paper, and the filtrates were centrifuged for 30 min at 3500 rpm. The supernatant was pipetted out and filtered through Whatman No. 1 filter paper. The filtered extracts were used for GC-MS analysis [7].

GC-MS Analysis of Both Callus and Micropropagated Plant Extracts: Aqueous and Methanol extracts of both callus and leaves of *in vitro* developed plants of *J. wynaadensis* were subjected to GC-MS analysis.

GC-MS Condition for Both Callus Extracts and Leaf Extracts of Micropropagated Plant: GC-MS analysis was performed using a Shimadzu GCMS-QP2010S System. Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Restek RTX-5 Capillary column (length 30m × 0.25mm ID) and 0.25µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. The oven temperature was programmed from 60 °C to 310 °C at 10 °C/min and a hold for 10 min. Helium was used as carrier gas at flow 1mL/min. The injector temperature was 250 °C, injection size 1uL neat, with split ratio 1:10. Ion source temperature was 200 °C. The interface and MS ion source were maintained at 320 °C and 320 °C, respectively. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 500 Da. Total GC running time was 40 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Data handling was done using GC-MS solution software. The identification of compounds was based on comparison of their mass spectra with those of NIST 5, NIST 10 and WILEY Libraries [8].

RESULTS AND DISCUSSION:

Callus Induction and Micropropagation: Induction of Callus Culture: Callus was obtained from stem explants. MS media supplemented with 2mg/L 2, 4-D found to be suitable for obtaining callus (**Fig. 1a**).

Micropropagation: After 1 week of inoculation of nodal explants on hormone free MS basal media, nodal bud and shoot growth was observed. Then after 4 weeks it was transferred to fresh medium supplemented with different concentration of BAP. 2-3 shoots with 1 inch height were obtained in medium with 1mg/L BAP, but MS medium with 4mg/L BAP concentration showed many shoots with less height (stunted growth) (**Fig. 1b**). MS medium with 1mg/L BAP was further used for 4 to 5 cycles to obtain more multiple shoots (**Fig. 1c**). After obtaining sufficient number of shoots, the cultures were transferred to fresh hormone free MS medium for shoot elongation. The shoots were increased in height after 4 weeks (**Fig. 1d**). The leaves obtained from elongated shoots were used for extraction.

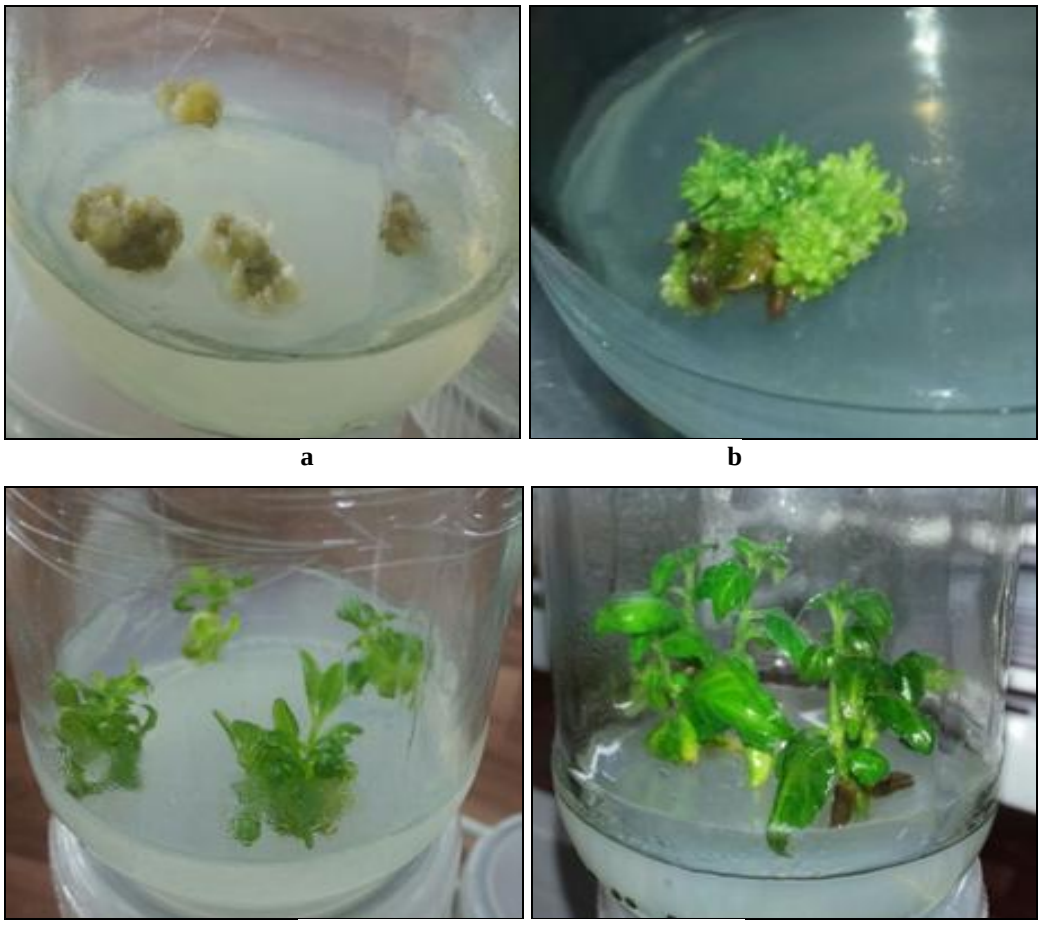
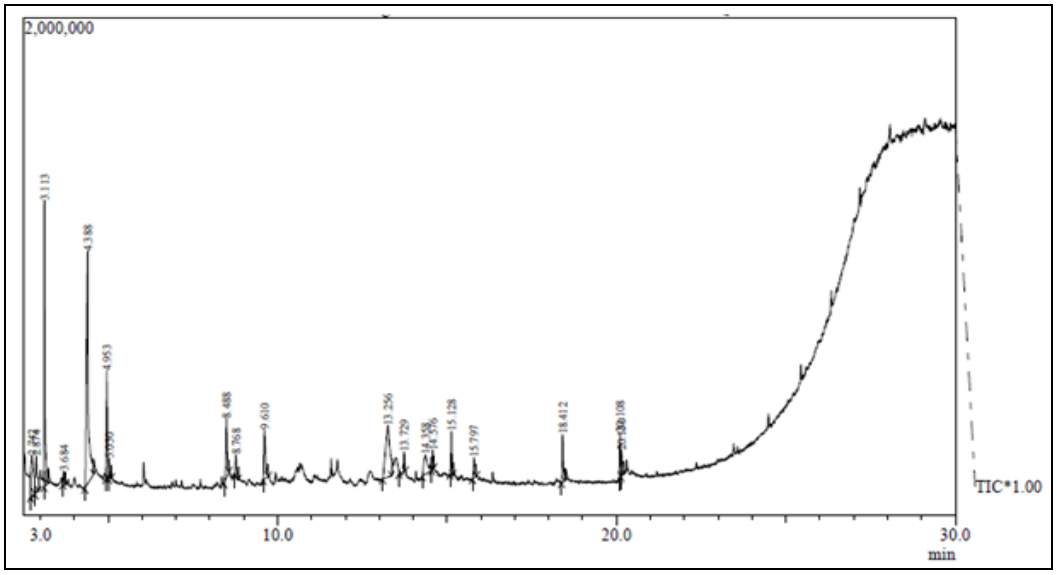


FIG. 1: INDUCTION OF CALLUS AND *IN VITRO* PROPAGATION OF *JUSTICIA WYNAADENSIS*

a. Induction of callus from stem explants on MS medium with 2mg/L 2,4-D.
b. Multiple shoot proliferation and stunted growth was observed at 4mg/L BAP in MS medium.
c. Multiple shoot proliferation from nodal explants on MS Medium supplemented with 1mg/L BAP.
d. Shoots on hormone free MS medium.

Phytochemical Analysis by GC-MS: The presence of various phytocompounds in callus and *in vitro* plant leaf extracts of *Justicia wynaadensis* were revealed by GC-MS analysis (**Fig. 2** and **Table 1, 2, 3, 4**). The aqueous callus extract contained 20 phytocompounds, and methanol callus extract showed presence of 41 phytocomponents. The aqueous extract of *in vitro* leaf revealed presence of 17 phytocompounds and methanol extract of *in vitro* leaf got resolved in to 41 peaks of which 38 compounds were identified.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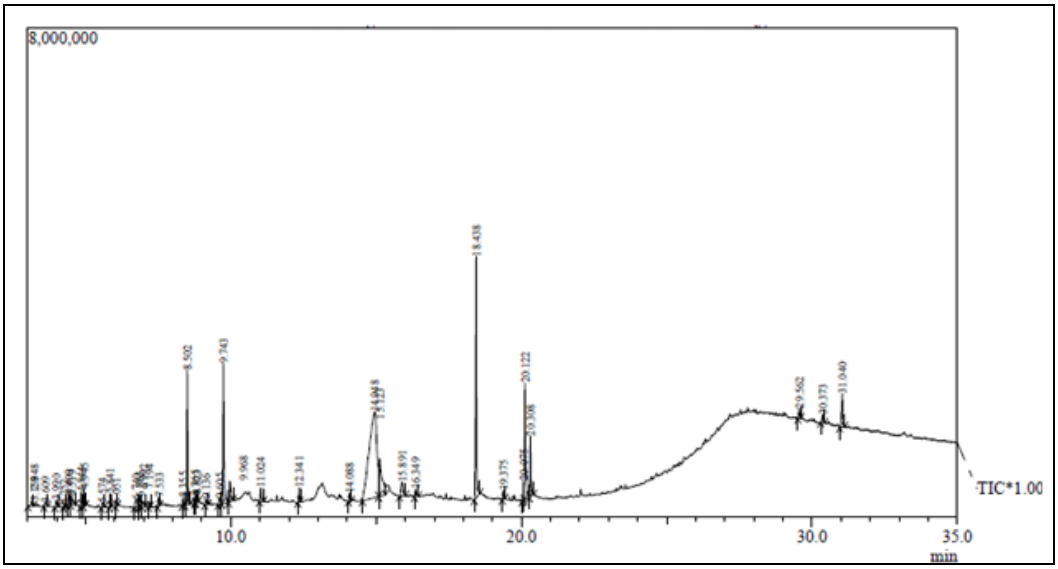
a


8,000,000
TIC*1.00
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30.0
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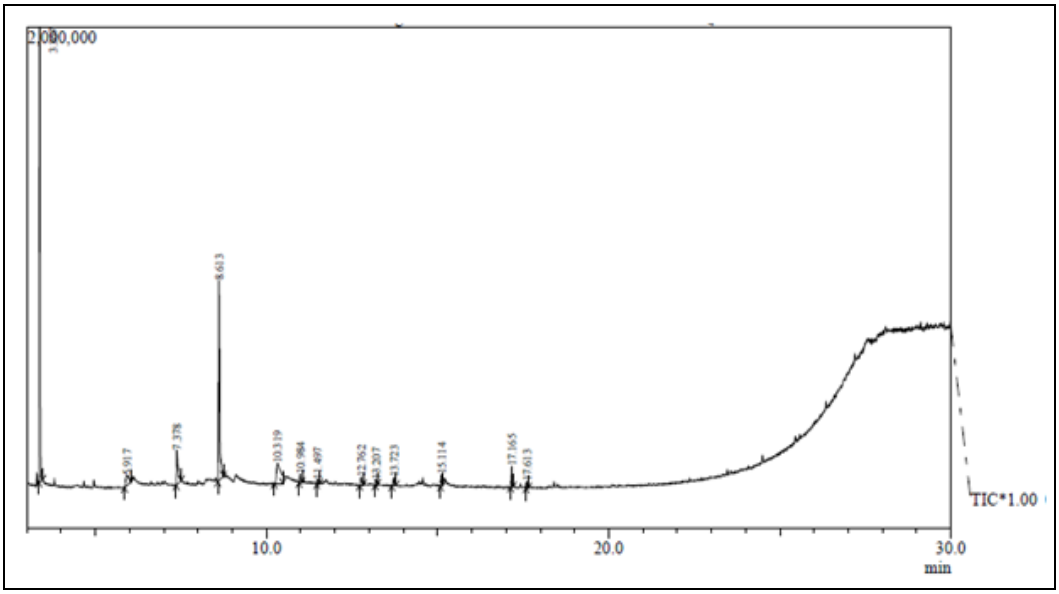
b


TIC*1.00
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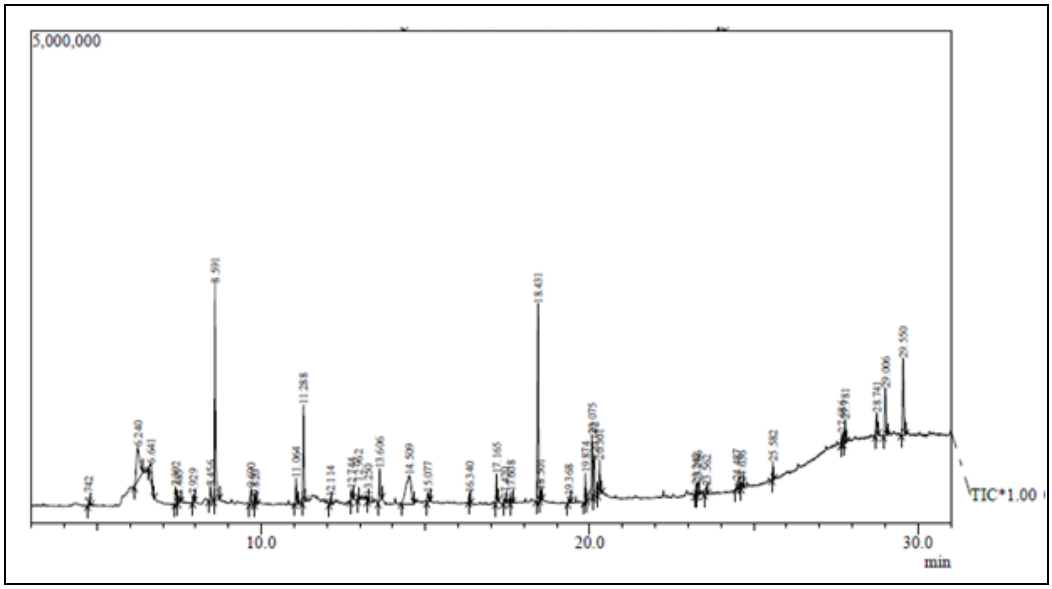
c



d

FIG. 2: GC MS CHROMATOGRAM OF CALLUS AND LEAF EXTRACTS OF *IN VITRO* PLANTS OF *JUSTICIA WYNAADENSIS*

a. GC-MS chromatogram of aqueous callus extract
b. GC-MS Chromatogram of Methanol callus extract
c. GC-MS Chromatogram of aqueous *in vitro* leaf extract
d. GC-MS Chromatogram of Methanol *in vitro* leaf extract

TABLE 1: COMPOUNDS IDENTIFIED IN THE AQUEOUS CALLUS EXTRACT OF *JUSTICIA WYNAADENSIS*

Sl. No.	R. Time	Peak Area %	Name of the Compound
1	2.276	8.89	2-Propanone, 1-hydroxy-
2	2.742	5.45	Propanoic acid, 2-methyl-
3	2.874	3.33	1-Penten-3-one
4	3.113	12.55	2,3-Butanediol
5	3.684	0.84	iso-Valeric Acid
6	4.388	24.5	(S)-2-Hydroxypropanoic acid
7	4.953	5.54	2,3-Dimethyl-Aziridine
8	5.03	1.04	6-Oxa-bicyclo[3.1.0]hexan-3-one
9	8.488	4.21	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
10	8.768	1.23	N-(Benzylidene)-2,2-dimethylcyclopropanecarbonitrile
11	9.61	4.21	1,2-Cyclohexanediol
12	13.256	11.43	L-Norleucine, 6-Chloro-2-Ethyl-
13	13.729	1.18	Phenol, 2-methoxy-4-(2-propenyl)-, acetate
14	14.358	4.01	.beta.-D-Glucopyranoside, methyl
15	14.576	1.04	1,2-Benzenedicarboxylic acid, diethyl ester
16	15.128	2.08	4-Allyl-1,2-diacetoxybenzene
17	15.797	1.54	.alpha.-D-Glucopyranoside, methyl
18	18.412	2.81	Hexadecanoic acid
19	20.108	2.43	Oleic Acid
20	20.15	1.71	Trichloroacetic acid, undec-2-enyl ester

TABLE 2: COMPOUNDS IDENTIFIED IN THE METHANOL CALLUS EXTRACT OF *JUSTICIA WYNAADENSIS*

Sl. No.	R. Time	Peak Area %	Name of the Compound
1	2.251	0.97	2-Propanone, 1-hydroxy-
2	2.848	0.58	2-Propyn-1-ol
3	3.159	0.38	Acetic acid, anhydride
4	3.609	0.41	2-Furancarboxaldehyde
5	3.99	0.5	2-Furanmethanol
6	4.252	0.21	2-Propenamide
7	4.409	0.79	(S)-2-Hydroxypropanoic acid
8	4.519	0.77	2-Propanone, 1,3-dihydroxy-
9	4.844	0.67	2(3H)-Furanone, dihydro-
10	4.945	0.8	2,3-Dimethyl-Aziridine

11	5.574	0.33	5 Methyl Furfural
12	5.841	0.48	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
13	6.051	0.13	2-Hydroxy-gamma-butyrolactone
14	6.74	1.14	Monomethyl malonate
15	6.865	0.58	Oxirane, phenyl-
16	6.992	0.56	Acetic acid, 2-(5-aminotetrazol-1-yl)-, ethyl ester
17	7.194	0.2	2,5-Dimethyl-4-hydroxy-3(2H)-furanone
18	7.533	0.75	Cyclopentane, 1-acetyl-1,2-epoxy-
19	8.355	0.47	2-acetyl-2-hydroxy-.gamma.-butyrolactone
20	8.502	5.81	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
21	8.765	0.36	Acetamide, N-(3-oxo-4-isoxazolidinyl)-,
22	8.823	0.41	Benzoic Acid
23	9.136	0.47	5-Methyl-2-ethoxy-3,4-dihydro-2H-pyran
24	9.605	0.51	3(2H)-Furanone, dihydro-5-isopropyl-
25	9.743	8.08	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
26	9.968	1.77	1,2,3-Propanetriol, monoacetate
27	11.024	0.92	cis-dimethyl morpholine
28	12.341	0.5	Propylphosphonic acid, fluoroanhydride, 4-methylcyclohexyl ester
29	14.088	0.42	Dodecanamide, N,N-bis(2-hydroxyethyl)-
30	14.948	35.66	.beta.-D-Glucopyranoside, methyl
31	15.123	6.19	4-Allyl-1,2-diacetoxybenzene
32	15.891	1.57	.beta.-D-Glucopyranoside, methyl
33	16.349	0.48	Tetradecanoic acid
34	18.438	11.83	Hexadecanoic acid
35	19.375	0.36	Heptadecanoic acid
36	20.075	0.71	1,E-11,Z-13-Octadecatriene
37	20.122	6.8	Octadec-9-Enoic Acid
38	20.308	2.77	Octadecanoic acid
39	29.562	0.67	.gamma.-Sitosterol
40	30.373	0.55	4,22-Stigmastadiene-3-one
41	31.04	2.45	9, 19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)-

TABLE 3: COMPOUNDS IDENTIFIED IN THE AQUEOUS EXTRACT OF *IN VITRO* LEAF OF *JUSTICIA WYNAADENSIS*

Sl. No.	R. Time	Pea Area %	Name of the Compound
1	2.075	3.86	1-Methyldecylamine
2	2.206	43.55	Acetic acid
3	2.262	4.37	2-Butanone, 3-hydroxy-
4	2.55	1.2	Silanediol, dimethyl-
5	3.367	19.69	2,3-Butanediol, [R-(R*,R*)]-
6	5.917	1.8	1,2,3-Propanetriol
7	7.378	3.05	2-Pyrrolidinone
8	8.613	13.6	2-Propenoic acid, 3-(2-hydroxyphenyl)-, (E)-
9	10.319	4.69	2,3-Butanedione
10	10.984	0.5	Phenol, 4-ethenyl-2-methoxy-
11	11.497	0.26	Phenol, 2,6-dimethoxy-
12	12.762	0.66	2H-1-Benzopyran-2-one
13	13.207	0.25	2,4,6,(1H,3H,5H)-Pyrimidinetrione, 5-acetyl-
14	13.723	0.65	Phenol, 2-methoxy-4-(2-propenyl)-, acetate
15	15.114	0.58	4-Allyl-1,2-diacetoxybenzene
16	17.165	0.94	Neophytadiene
17	17.613	0.36	Citronellyl butyrate

TABLE 4: COMPOUNDS IDENTIFIED IN THE METHANOL EXTRACT OF *IN VITRO* LEAF OF *JUSTICIA WYNAADENSIS*

Sl. No.	R. Time	Peak Area %	Name of the Compound
1	4.742	0.33	2(3H)-Furanone, dihydro-
2	6.24	7.23	L-(-)-Menthol
3	6.641	2.17	1,2,3-Propanetriol
4	7.392	1.41	2-Pyrrolidinone
5	7.485	0.61	2-Hexanone, 3-methyl-4-methylene-
6	7.929	0.45	1H-Pyrrole, 2,5-dihydro-
7	8.456	1.08	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-

8	8.591	13.75	2-Propenoic acid, 3-(2-hydroxyphenyl)-, (E)-
9	9.690	1.39	1,3-Benzodioxole, 5-(2-propenyl)-
10	9.820	0.63	l-Menthyl acetate
11	11.064	1.66	Eugenol
12	11.288	6.17	Isoeugenol
13	12.114	0.43	trans-Caryophyllene
14	12.744	0.92	2H-1-Benzopyran-2-one
15	12.962	0.31	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-
16	13.25	0.25	.alpha.-selinene
17	13.606	1.95	Phenol, 2-methoxy-4-(2-propenyl)-, acetate
18	14.509	9.55	.alpha.-D-Glucopyranoside, methyl
19	15.077	0.91	4-Allyl-1,2-diacetoxybenzene
20	16.34	0.43	Tetradecanoic acid
21	17.165	1.41	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-
22	17.42	0.45	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
23	17.608	0.58	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
24	18.431	11.94	Hexadecanoic acid
25	18.501	0.57	
26	19.368	0.43	Docosanoic acid
27	19.874	1.66	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-
28	20.075	6.08	9,12-Octadecadienoic acid, methyl ester, (E,E)-
29	20.142	4.78	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-
30	20.301	2.24	Octadecanoic acid
31	23.242	0.77	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
32	23.286	1.23	4H-1-Benzopyran-4-one, 2,3-dihydro-5,7-dihydroxy-2-phenyl-, (S)
33	23.562	0.54	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
34	24.487	0.45	
35	24.636	0.42	Z,Z-4,15-Octadecadien-1-ol acetate
36	25.582	0.84	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-,
37	27.686	0.83	
38	27.781	1.57	Vitamin E acetate
39	28.741	1.71	Ergost-5-en-3.Beta.-ol
40	29.006	3.47	Stigmasta-5,22-dien-3-ol, (3.beta.,22E)-
41	29.55	6.41	.gamma.-Sitosterol

Medicinal important and bioactive phytocomponents found in all the four extracts and its biological activity and pharmaceutical importance: 2-Propanone, 1-hydroxy- (synonym- Acetol) found in both aqueous and methanol extract of callus is used as analgesic and as flavoring agent. 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl found in aqueous and methanol extracts of callus and in methanol extract of *in vitro* leaf was found to have antimicrobial, anti-inflammatory, anti-proliferative activity [9]. Hexadecanoic acid (synonym - Palmitic acid) was present in aqueous callus extract, methanol callus extract and in methanol extract of *in vitro* leaf, and it is reported that it is an antioxidant, antimicrobial, 5- alphareductase- inhibitor, anti-fibrinolytic, hemolytic, antialopecic, lubricant, nematicide and used as flavoring agent [8]. Oleic acid found in the aqueous callus extract, is used in the preparation of oleates, lotions, and as a pharmaceutical solvent [10]. 2- Propanone, 1, 3- dihydroxy- (synonym- Dihydroxy-acetone) and Benzoic acid were identified in methanol extract of callus. Dihydroxyacetone acts as a sun screening agent in combination with naphthoquinones. Benzoic acid is antifungal and antimicrobial agent and it is used as food preservatives. Tetradecanoic acid (synonym - Myristic acid) was present in methanol extracts of both callus and *in vitro* leaf. It is an antioxidant, hypercholesterolemic, cancer-preventive, lubricant, used in cosmetics, acts as nematicide [8]. Heptadecanoic acid (synonym - Margaric acid) which was identified in methanol callus extract has antioxidant property [8]. From methanol extracts of callus and *in vitro* leaf Octadecanoic acid and gamma. Sitosterols (synonym - Sitosterol) were identified.

Octadecanoic acid which is also called as Stearic acid used in cosmetic, lubricant, perfumery, and as flavoring agent, was reported as hypo-cholesterolemic [8, 9]. Sitosterols are used to treat hyperlipidemias.

In aqueous extract of *in vitro* leaf acetic acid (synonym-Ethylic acid) and 2-Butanone, 3-hydroxy-, were found. Acetic acid has antibacterial and antifungal properties and 2-Butanone, 3-hydroxy-, is used in food flavoring and for fragrance. In aqueous and methanol extracts of *in vitro* leaf 1, 2, 3-Propanetriol (synonym-Glycerol) was identified, it is used as a solvent, emollient, pharmaceutical agent, and sweetening agent. It is also reported as diuretic, hyperosmotic, Laxative, stool softener [10]. 2- Propenoic acid, 3- (2-hydroxyphenyl)-, (E)- or Cinnamic acid dihydro and 2H- 1- Benzopyran- 2- one (synonym - Coumarin) were identified in aqueous and methanol extracts of *in vitro* leaf. Activity of Cinnamic acid dihydroare antibacterial, anesthetic, antiinflammatory, antimutagenic, antispasmodic, cancer preventive, choleretic, dermatitigenic, fungicide, laxative, Aldose-Reductase-Inhibitor, Lipoxygenase Inhibitor, Tyrosinase Inhibitor, vermifuge, flavor. Coumarin acts as cancer-preventive and used as flavours [8]. It is also reported as antimutagenic, anticoagulant, anti-inflammatory and bacteriostatic [11-14].

Neophytadiene found in aqueous extract of *in vitro* leaf, has anti-inflammatory property [15]. It is also reported to be antioxidant, antipyretic, analgesic and antimicrobial [16]. L-(-)-Menthol; 1, 3-Benzodioxole, 5- (2-propenyl); l- Menthyl acetate; Eugenol; Isoeugenol; trans-Caryophyllene; 2- Hexadecen- 1-ol, 3, 7, 11, 15-tetramethyl-, [R-[R*,R*-(E)]]-; Docosanoic acid; 2, 6, 10, 14, 18, 22- Tetra-cosahexaene, 2, 6, 10, 15, 19, 23- hexamethyl-; Vitamin E acetate; Ergost- 5- en- 3. Beta. -ol; Stigmasta- 5, 22- dien- 3- ol, (3.beta, 22E), were present in methanol extract of *in vitro* leaf.

L-(-)-Menthol is used to treat sore mouth, sore throat, occasional minor irritation, pain and cough associated with cold. It produces local analgesic or anesthetic effect and used as decongestants. Used in mouth washes, toothpaste, shampoos and perfumes [10].

1, 3-Benzodioxole, 5-(2-propenyl)-, (synonym-Safrole) is reported as cancer preventive and anti-hepatoma [17, 18]. l- Menthyl acetate is an anticancer, anticarcinomic, antioxidant, antitumor and cytotoxic agent [18]. Eugenol is antiseptic, anti-inflammatory, and antimicrobial. Both Eugenol and Isoeugenol are used in perfumeries, flavourings, essential oils and in medicine (as local antiseptic and analgesic) and is an antioxidant [19, 10]. Trans-Caryophyllene is a Catechol- O- Methyl Transferase inhibitor, anti-inflammatory agent, analgesic, antipyretic, and has platelet-inhibitory actions [18, 10]. 2- Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*, R*-(E)]]-, (synonym Phytyl Acetate/Phytol) is cancer-preventive [8], antimicrobial, anti-inflammatory, diuretic [20]. Docosanoic acid is used in cosmetics in hair conditioners and moisturizers [10]. 2, 6, 10, 14, 18, 22- Tetracosa-hexaene, 2,6,10,15,19,23-hexamethyl-, (synonym - Squalene) is an antibacterial, antioxidant, cancer-preventive, antitumor, immunostimulant, used in perfumery and in sunscreen [8], Squalene has also been reported to act as chemopreventive agent [21]. Vitamin E acetate (synonym-Vitamin E; Alpha-Tocopherol) act as an antiaging, antialzheimeran, antidermatitic, antidiabetic, antioxidant, antitumor, cancer preventive, hypocholesterolemic, immuno-stimulant [8]. Ergost- 5- en-3. Beta.- ol (synonym-Campesterol) is an antioxidant, hypocholesterolemic [8] and cancer preventive [22]. Stigmasta-5, 22-dien-3-ol, (3.beta. 22E)-, (synonym-Stigmasterol) is an antihepatotoxic, antiviral, antioxidant, hypocholes-terolemic [8] and cancer preventive [22].

CONCLUSION: The present study revealed the presence of phytocomponents with various therapeutically useful properties from the aqueous and methanol extracts of callus and *in vitro* propagated leaf of *J. wynaadensis* by GC-MS analysis. The study has shown that extracts of *in vitro* regenerated *J. wynaadensis* is rich in bioactive secondary metabolites. *In vitro* propagation technique helps to obtain more number of plants in short period, from which potential bioactive phytochemicals can be isolated and used in pharmaceutical industries.

ACKNOWLEDGEMENT: The authors wish to thank gratefully to PES institute of Technology, Bangalore for providing facility to carry out this research work.

CONFLICT OF INTEREST: The authors do not have any conflict of interest.

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How to cite this article:
Vandana CD, Shanti KN and Shantha SL: GC-MS analysis of callus and leaf extracts of *in vitro* propagated plants of *Justicia wynaadensis* (nees) T. Anderson. Int J Pharm Sci & Res 2018; 9(2): 535-43. doi: 10.13040/IJPSR.0975-8232.9(2).535-43.