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FTIR AND GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS IN ETHANOL EXTRACT OF TOBACCO CALLUS (*NICOTIANA TABACUM* L.)

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ABSTRACT

Standardization of protocol for callus initiation and regeneration of *Nicotiana tobacum* (Solanaceae) was achieved. Indirect regeneration, multiplication of plantlets were observed from the leaf explant on MS medium augumented with BAP (2.22 µm) and NAA (1.14 µm). The higher percentage of callus forming shoots (99 %) and highest number of shoots during subculture (25) was obtained on the same callus induction medium. Preliminary phytochemical analysis of ethanol leaf callus extract showed the presence of alkaloids, steriod, tannins, phenol, glycosides and triterpenoids. The FTIR(Fourier Transform Infrared spectroscopy) analysis showed 8 major peaks confirmed the presence of alkyl halides, aliphatic amines, aromatic amines, nitro compounds, alkenes and alkyls groups. GCMS results revealed the presence of 47 compounds. Among these 7 high peak and 11 biologically active compounds.

KEYWORDS: Indirect regeneration, FTIR, GCMS, *Nicotiana tobacum*, Callus.

INTRODUCTION

Medicinal plants are a source of great economic value all over the World. Nature has been showed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Herbal medicine is still the main stay of about 75-80% of the World population, and the major part of traditional therapy involves the use of plant extract and their active constituents.[1] Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades advances in phytochemistry and in

identification of plant compounds effective against certain diseases have renewed the interest in herbal medicines.

Micropropagation is a advantageous technique for the conservation and amplification of rare or endangered plants with medicinal value. The callus produced, can be utilized directly to regenerate plantlets or to extract or manipulate some primary and secondary metabolite.[2] There are various advantages of a callus culture system over the conventional cultivation of whole plants. Useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions. The callus of any plants, could easily be multiplied to yield their specific metabolites, automated control of callus growth and rational regulation of metabolite processes would reduce labor costs and improve productivity also the organic substances are extractable from callus cultures.[3]

Fourier transform infrared spectrometry (FT-IR) is a physico-chemical analytical technique that does not resolve the concentrations of individual metabolites but provides a snapshot of the metabolic composition of a tissue at a given time.[4] FT-IR has been exercised to identify the concrete structure of certain plant secondary metabolites.[5,6]

Gas chromatography has a very wide field of applications the separation and analysis of multi component mixtures such as essential oils, hydrocarbons and solvents. The use of the flame ionization detector and the electron capture detector gas chromatography can quantitatively determine materials present at very low concentrations. Because of its simplicity, sensitivity, and effectiveness in separating components of mixtures, gas chromatography is one of the most important tool in chemistry.[7]

Nicotiana is a genus of herbaceous plants and shrubs of the family Solanaceae, that is indigenous to the Americas, Australia, South West Africa and the South Pacific. Various *Nicotiana* species, commonly referred to as tobacco plants, are cultivated as ornamental garden plants. *Nicotiana tabacum* (Tobacco) is a stout herbaceous plant in the Solanaceae (nightshade family) that originated in the tropical America and now cultivated Worldwide as the primary commercial source of tobacco, which is smoked or chewed as a drug for its mild stimulant effects. The World Health Organization (WHO) estimates that around 1 billion people worldwide smoke tobacco- around 14% of the global population - making tobacco a major cash crop in many places.

Some other activities reported for *Nicotiana tabacum* are: analgesic activity, anesthetic activity, angiogenesis inhibition, antibacterial activity, anti convulsant activities, anti-estrogenic effect, antifungal activity, antiglaucomic activity, antioxidant activity, antistress effect, antiviral activity, aromatase inhibition, arrhythmogenic effect, carcinogenic activity, Nicotine for the treatment of Alzheimer disease, Parkinson disease, depression and anxiety, schizophrenia, attention deficit hyperactivity disorder (ADHD), pain, and obesity. Leaves also contain glucosides, tahacinin, tahacilin and *iso*quercitrin, 1-quinic, chlorogenic, caffeic and oxalic acids. They also contain terpenic and carcinogenic substances.[8] Keep this in view, the current study was planned to standardize the media for callus induction, regeneration and to analyze the functioned groups through FTIR and to find out the bioactive compounds by GCMS in callus extract of Tobacco.

MATERIALS AND METHODS

Source of Plant materials: The tobacco seeds were obtained from local market, Sathyamangalam, Coimbatore for this study.

Seed treatment: The seeds were treated with 70% ethyl alcohol for 1 min, and then rinsed with sterile water for three times. The seeds were then surface sterilized in 0.12% mercuric chlorite solution for 10 min and was followed by three washes in succession with sterile water prior to inoculation.

In vitro seed germination

Sterilized seeds were inoculated on Murashige andSkoog's (MS) basal medium. The cultures were incubated in 25 ± 2°C under 16/8 hr (light and dark) condition for 30 days period to observe rate of germination from 10^{th}, 20^{th}, 30^{th}, days.

Culture media employed and their composition

MS[9] basal medium in full strength (MS) along with cytokinin (BAP, TDZ and KN) and auxin (NAA) at various concentration was employed in the present study.

Culture conditions

All the cultures were maintained in the culture room at a temperature of 25±2°C and relative humidity of 65-70%. The cultures were kept under white light at intensity of 3000 Lux provided from white fluorescent lamps (Philips, India) with 12 hours photoperiodic duration.

Callus initiation and shoot proliferation

Leaf explants from *in vitro* grown plants were used as primary explants. The explants were cultured on MS medium supplemented with various concentrations of growth regulators (BAP, NAA, TDZ and KN). Twenty explants were used for each culture. The percent of explants responding for callus induction, shoot formation, nature of callus and number of days taken for callus induction were recorded after 40 days. In the subsequent subcultures, the callus and other parts obtained *in vitro* cultures were harvested and used as explants. Sub culturing was carried out at regular interval of 15-20 days.

Analysis of bioactive compounds: The present investigations on ethanol extract of *Nicotiana tabacum in vitro* callus were carried out through phytochemical, FTIR and GC-MS analysis.

Preparation of plant extracts: The powdered callus of tobbaco was successively extracted using 50 ml of ethanol by using the Soxhlet extractor for 8-10 hrs.[10] The extract was filtered through Whatmann No.1 filter paper to remove all undissolved matter including cellular materials and other constitutions that are insoluble in the extraction solvent.

Analysis of functional group: The present investigations on leaf callus extract of *Nicotiana tabacum* was carried out through FT-IR.

GC-MS analysis

Plant sample extraction: The fresh weight of the callus was measured and it was air dried and powdered and dry weight were measured and stored in room temperature. Five grams of powder was soaked in 10ml of ethanol overnight and then filters through Whatmann filter paper No.1 along with 2gms sodium sulphate to remove the sediments and traces of water in the filtrate. The filtrate is then concentrated by bubbling nitrogen gas in to solution and reduces the volume to one ml. The extract contains both polar and non-polar phytocomponents of the plant material and plant extract is injected in the Gas chromatography-Mass Spectrometer.

OBSERVATION

In vitro seed germination percentage of *N. tabacum* in different media conditions are shown in table 1. It was observed that MS basal salt media have 100 % of germinated seeds in thirty days culture. The germination percentage was significantly reduced when MS media addition

with BAP 0.5 mg/l on thirtieth day as maximum 85% of germination. In all treatmentsthe germination rate was positively correlated with germination percentage. *In vitro* seed germination have reduced when the addition of growth regulators in MS media up to 85 %.

Table - 1. Germination percentage of *Nicotiana tabacum* seeds under different treatment

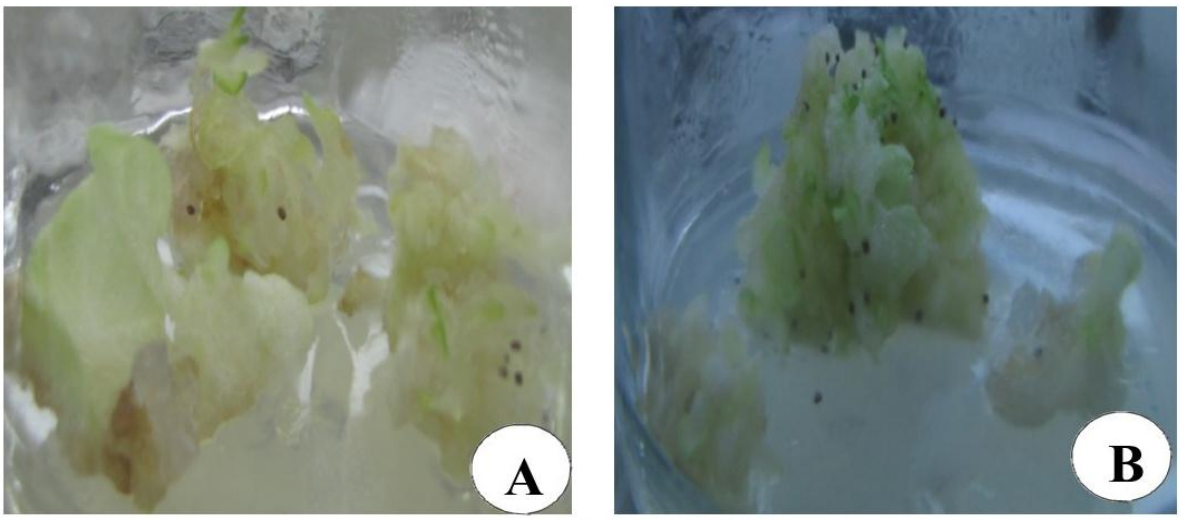
S. No	Treatments	Percentage of germination		
		10 days	20 days	30 days
1	MS Basal	95.48 ± 0.14	99.99 ± 0.08	100.05 ± 0.07
2	MS + BAP (0.5 mg/l)	80. 01 ± 0.13	85.10 ± 0.13	85.12 ± 0.04
3	MS + BAP (1.0 mg/l)	70.01 ± 0.13	75.03 ± 0.14	76.04 ± 0.07
4	MS + NAA (0.5 mg/l)	70.08 ± 0.12	72.35 ± 0.17	73.09 ± 0.06
5	MS + NAA (1.0 mg/l)	68.03 ± 0.12	69.01 ± 0.13	69.13 ± 0.09
6	MS + TDZ (0.5 mg/l)	72.05 ± 0.14	73.08 ± 0.10	75.07 ± 0.02
7	MS + TDZ (1.0 mg/l)	65.05 ± 0.12	67.03 ± 0.09	68. 06± 0.04
8	MS + KN (0.5 mg/l)	63.10 ± 0.15	65.05 ± 0.11	66.15 ± 0.05
9	MS + KN (1.0 mg/l)	58.03 ± 0.13	60.00 ± 0.09	62.11 ± 0.08

Callus culture

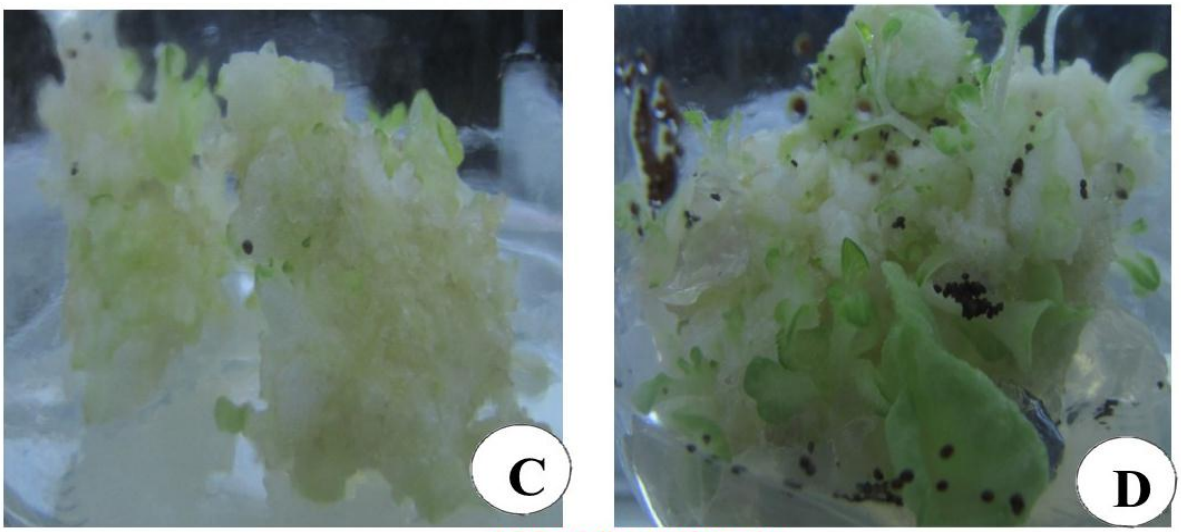
The combination of BAP and NAA induced an excellent amount of callus from leaf of *N. tabacum* and the morphology of the callus was green, friable, and nodular in nature. Best growth of callus however occurred on MS + BAP (4.44 µM) + NAA (1.14 µM). The other concentrations of BAP was also effective but not at the level of previous combination. Callus started at the cut ends or along the entire surface after 8 days of culture, and after 15 to 20 days the entire segment turned into a mass of green, soft, and friable callus (Plate 1). Here the response percentage was 99 and it was followed by BAP (2.22 µM) + NAA (1.14 µM) with 96%. In BAP + KN (4.44 + 1.34 µM) combination also the response percentage was 98 (Table 2).

All other combination also induced shoot from callus but the higher percentage of callus forming shoots (99%) and highest number of shoots during subculture (25) was obtained in the combination of BAP + NAA (2.22 + 1.14 µM). This is followed by BAP + NAA (2.22 + 1.14 µM) with 98% shoot induction. Among other combinations the BAP (4.44 µM) alone induced 88% shoots (Table 2). Multiple Shoot formation from the callus occurred on the same medium containing BAP+NAA (4.44 µM + 1.14 µM) or when calli was transferred to fresh medium. During subculture 25.00 ± 0.09 shoots were induced from explant within 20 to 25 days. The shoots elongated and grew and developed many leaves (Plate 1).

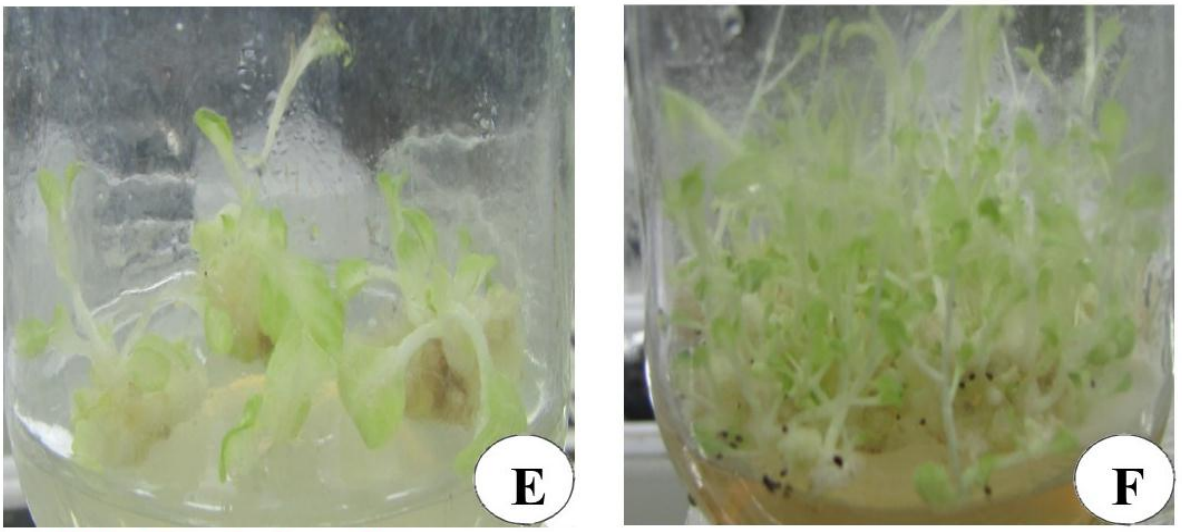
PLATE - 1. Callus induction and multiple shoot formation of *Nicotiana tabacum*



A & B - Callus formation from leaf



C & D – Callus multiplication



E - Shoot formation from callus

F - Multiple shoot formation from callus sub culture

Table 2: Effect of MS medium and different concentration and combination of BAP, TDZ, KN and NAA on callus induction and shoot formation in leaf explant of *Nicotiana tabacum.*

S. No	MS medium				Response%	Leaf producing callus	Nature of the callus	%Callus forming shoots	Shoot number – Sub culture
	BAP µM	NAA µM	TDZ µM	KN µM					
1	**2.22**	**1.14**	-	-	**96.23 ± 0.07**	++	**Green friable**	**98.00 ± 0.11**	**19.99 ± 0.11**
2	**4.44**	**1.14**	-	-	**99.45 ± 0.13**	++	**Friable and dark green**	**99.03 ± 0.12**	**25.00 ± 0.09**
3	6.66	1.14	-	-	95.40 ± 1.12	++	Green friable	97.04 ± 0.09	15.85 ± 0.05
4	8.88	1.14	-	-	94.38 ± 0.10	++	Green friable	95.05 ± 0.11	10.15 ± 0.12
5	11.11	1.14	-	-	90.45 ± 0.09	++	Green friable	80.04 ± 0.14	8.78 ± 0.13
6	2.22	-	1.16	-	81.23 ± 0.12	++	Green friable	82.11 ± 0.06	18.65 ± 0.05
7	4.44	-	1.16	-	85.20 ± 0.16	++	Green friable	85.12 ± 0.04	17.80 ± 0.11
8	6.66	-	1.16	-	82.51 ± 0.13	++	Green friable	78.08 ± 0.13	15.35 ± 0.09
9	8.88	-	1.16	-	80.14 ± 0.07	++	Green friable	77.04 ± 0.09	11.70 ± 0.04
10	11.11	-	1.16	-	79.21 ± 0.04	+	Green friable	70.07 ± 0.06	9.55 ± 0.03
11	2.22	-	-	-	82.42 ± 0.13	++	Green friable	90.05 ± 0.07	22.80 ± 0.04
12	4.44	-	-	-	83.33 ± 0.11	++	Green friable	88.12 ± 0.12	23.45 ± 0.09
13	6.66	-	-	-	79.41 ± 0.09	+	Green friable	80.06 ± 0.04	20.65 ± 0.07
14	8.88	-	-	-	77.30 ± 0.05	+	Green friable	70.09 ± 0.01	15.70 ± 0.05
15	11.11	-	-	-	75.43 ± 0.06	+	Green friable	69.03 ± 0.03	10.25 ± 0.11
16	2.22	-	-	1.34	95.18 ± 0.04	++	Friable and dark green	88.07 ± 0.07	15.80 ± 0.12
17	4.44	-	-	1.34	98.39 ± 0.14	++	Friable and dark green	85.12 ± 0.11	13.55 ± 0.09
18	6.66	-	-	1.34	93.25 ± 0.16	++	Green friable	82.06 ± 0.10	10.45 ± 0.05
19	8.88	-	-	1.34	92.22 ± 0.14	++	Green friable	75.03 ± 0.12	9.25 ± 0.06
20	11.11	-	-	1.34	90.41 ± 0.13	++	Green friable	72.08 ± 0.08	8.70 ± 0.07
21	Basal medium	-	-	-	-	-	-	-	-

Preliminary phytochemical analysis

Preliminary phytochemical analysis of ethanolic leaf callus extracts of *N. tabacum* are presented in the table 3. The phytochemical analysis showed the presence of alkaloids, steroids, tannins, phenolics, flavonoids, glycosides and triterpenoids (Table 3).

Table. 3: Preliminary phytochemical analysis of ethanolic callus extract of *Nicotiana tabacum*.

S. No	Secondary metabolites	Ethanolic extract
1	Alkaloids	+
5	Saponins	-
2	Steroid	+
8	Tannins	+
4	Phenol	+
6	Flavonoids	-
3	Glycosides	+
7	Triterpenoids	+
8	Reducing sugars	-

FT-IR Spectroscopy

The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The callus powder of *N. tabacum* passed with FT-IR and the functional groups of the components werebased on its peak ratio . The results of *N. tabacum* ethanolic leaf callus extract FT-IR analysis showed major peaks at 433.44, 454.99, 533.33, 1064.03, 1241.30, 1384.93, 1682.42 and 3289.35. This peaks confirmed the presence of alkyl halides, aliphatic amines, aromatic amines, nitro compounds, alkenes, alkyls respectively (Figure 1).

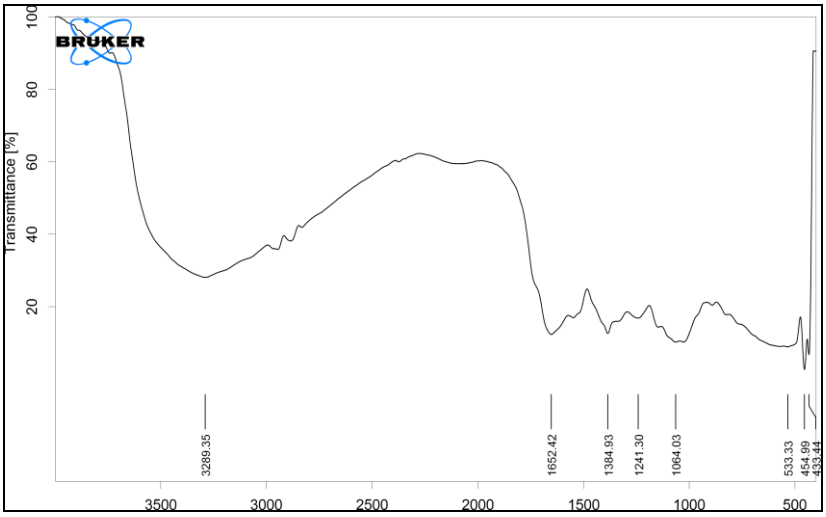


Figure. 1: Compounds identified in the callus extract of *Nicotiana tabacum* by FTIR.

GC-MS analysis

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractionations of the ethanolic leaf callus extract of *N. tabacum*. These compounds ware identified through mass spectrometry attached with GC. The results of the present study were tabulated in Table 4. The compound prediction is based on National Institute Standard and Technology Database. The results revealed that the presence of high peak compounds Phthalic acid, 6-ethyloct-3-yl 2-ethylhex (23.38%), 18-Nonadecenoic acid (10.42%), Z-11-Pentadecenol (8.86%), Pentadecanoic acid (6.06%), n-Hexadecanoic acid (2.76%),l-(+)-Ascorbic acid 2,6-dihexadecanoate (2.54%), Cyclononasiloxane, octadecamethyl (1.93%), The spectrum profile of GC-MS confirmed the presence of 47 major components with the retention time 34.436, 27.473, 27.399, 27.891, 23.624, 24.170, 29.905 respectively (Figure: 2).

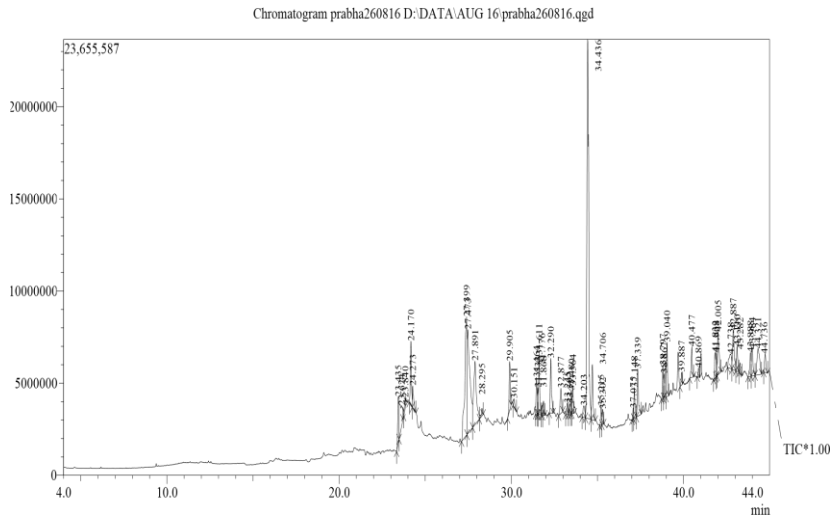


Figure. 2: Phytocomponents identified in ethanolic leaf callus extracts of *Nicotiana tabacum* by GCMS.

Table. 4: Phytocomponents identified in ethanolic leaf callus extracts of *Nicotiana tabacum* by GCMS.

S.NO	RT	Compound Name	Molecular Formula	Molecular Weight	Peak Area (%)	Bioactive uses
1	23.435	6-[1-(HYDROXYMETHYL)VINYL]-4	$C_{15}H_{24}O_2$	236	2.07	-
2	23.624	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.76	Antioxidant,Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor (Praveen kumar et al., 2010).
3	23.840	9-OCTADECENOIC ACID (Z)-	$C_{18}H_{34}O_2$	282	0.46	steroids and primer pheromone.
4	24.170	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	2.54	antioxidant, antiinflammatory and anti nociceptive,analgesic, antispasmodic and antibacterial properties (Okenwa U. Igwe 2014).
5	24.273	DOCOSANOIC ACID	$C_{22}H_{44}O_2$	312	0.92	Detergents and floor polishes(Renji R. Nair 2017)
6	27.399	Z-11-Pentadecenol	$C_{15}H_{30}O$	226	8.86	-
7	27.473	18-Nonadecenoic acid	$C_{19}H_{36}O_2$	296	10.42	insects as pheromones
8	27.891	Pentadecanoic acid	$C_{10}H_{22}O$	158	6.06	antibacterial and antifungal activities
9	28.295	TERT-BUTYL PALMITATE	$C_{11}H_{24}O$	172	0.37	-
10	29.905	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	666	1.93	-
11	30.151	5,5-Dibutylnonane	$C_{17}H_{36}$	240	0.33	-
12	31.464	Squalene	$C_{30}H_{50}$	410	1.28	Antibacterial,Antioxidant,Pesticide, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor
13	31.533	Geranylgeraniol	$C_{20}H_{34}O$	290	0.72	-
14	31.611	SOLANESOL	$C_{45}H_{74}O$	630	2.22	-
15	31.776	BACTERIOCHLOROPHYLL-C-STEA	$C_{52}H_{72}MgN_4O_4$	840	0.33	-
16	31.868	Dotriacontane	$C_{32}H_{66}$	450	0.48	Antimicrobial agent, hypercholesterolemic (Ngassoum *et al.*,2000)
17	32.290	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	740	2.27	-
18	32.877	NERYL LINALOOL ISOMER	$C_{20}H_{34}O$	290	1.21	-

19	33.245	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2	$C_{30}H_{50}O$	426	0.39	-
20	33.392	1-Heptacosanol	$C_{27}H_{56}O$	396	0.37	Nematicidal , anticancer, antioxidant and antimicrobial (Venkata Raman B 2012).
						-
21	33.480	HEXATRIACONTANE	$C_{36}H_{74}$	506	0.70	-
22	33.564	1,2-Benzenedicarboxylic acid, mono(2-e	$C_{16}H_{22}O_4$	278	1.11	-
23	34.203	ACETIC ACID, (TRIPHENYLPHOSPH	$C_{21}H_{19}O_2P$	334	0.74	-
24	34.436	Phthalic acid, 6-ethyloct-3-yl 2-ethylhex	$C_{26}H_{42}O_4$	418	23.38	-
25	34.706	Heptasiloxane, hexadecamethyl			2.50	-
26	35.215	1-Octacosanol	$C_{28}H_{58}O$	410	0.64	-
27	35.302	TRIACONTANE	$C_{30}H_{62}$	422	0.46	-
28	37.075	Cyclohexane, [6-cyclopentyl-3-(3-cyclo	$C_{25}H_{46}$	346	0.33	-
29	37.148	Pentacosane			1.06	-
30	37.339	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	888	2.23	-
31	38.797	Heptacosyl heptafluorobutyrate	$C_{31}H_{55}F_7O_2$	592	1.01	-
32	38.867	2-METHYLHEXADECANE	$C_{17}H_{36}$	240	0.69	-
33	39.040	Propanoic acid			2.09	-
34	39.887	Nerolidyl acetate			0.42	-
35	40.477	Tetrapentacontane	$C_{54}H_{110}$	758	1.11	-
36	40.869	GERANYL LINALOOL ISOMER			0.35	-
37	41.832	CYCLOPENTADECASILOXANE, TR	$C_{30}H_{90}O_{15}Si_{15}$	1110	1.05	-
38	41.908	Stigmasterol	$C_{29}H_{48}O$	412	0.74	Anti-tumor, Cancer preventive, inhibit intestinal cholesterol absorption. Antiinflammatory (Premlata Singariya et al., 2015).
39	42.005	Octacosyl pentafluoropropionate	$C_{31}H_{57}F_5O_2$	556	2.33	-
40	42.738	BUTANOIC ACID, 3,7-DIMETHYL-6	$C_{14}H_{26}O_2$	226	1.37	-
41	42.887	ERGOSTA-7,22-DIEN-3-OL, (3.BETA	$C_{28}H_{46}O$	398	2.30	lowering cholesterol
42	43.110	gamma.-Tocopherol	$C_{28}H_{48}O_2$	416	1.03	-
43	43.282	22E,24R)-24-ETHYLCHOLESTA-5,7,9	$C_{29}H_{44}O$	408	0.40	-

44	43.888	Heptadecane, 3-methyl-			1.08	-
45	43.984	Ursodeoxycholic acid			1.47	-
46	44.321	SILICONE OIL			2.51	-
47	44.736	alpha.-Tocopherol-.beta.-D-mannoside	$C_{35}H_{60}O_7$	592	0.88	-

DISCUSSION

The relative effectiveness of different physicochemical and plant growth regulators treatments in causing germination improvement is summarized in Table 1. In general, the seed germination rate was good in all the treatments, despite the growth regulator treatments have very positive effect on germination rate in *N. tabacum*. Seed germination was significantly different among growth regulator treatments. As a whole, MS basal medium obviously increased germination percentage. The results indicate that the MS basal medium induced 100% germination when compared to all other treatments. The effectiveness of MS basal medium on tabaco seed germination has also been reported.[11] The other treatment also induced seed germination but due to the effect of growth regulators the percentage was reduced when compared to MS basal medium. This maybe due to the adverse effect of growth regulators. This is in accordance with *Ceropegia pusila* seed germination.[12] Among the growth regulators the germination was high (85%) in BAP (0.5 mg/l), followed by BAP (1.0 mg/l) with 76% and 75% in TDZ (0.5 mg/l), 73% in NAA (0.5 mg/l). The least germination percentage (62%) was observed in KN (1.0mg/l). This results showed that among the growth regulators the effect of KN was more among the growth regulators.

The combination of BAP and NAA induced greater amount of callus from the leaf of *N. tabacum* and the morphology of the callus was green and friable and nodular in nature. The caulogenic effect of BAP along with NAA observed in the present study is in consonance with other reports.[13-15] Best growth of callus however occurred on MS + BAP (4.44 µM) + NAA (1.14 µM). Similar observation was reported in *N. tabacum*[16] *C. pusilla* from the cell layer explants[17] which indicate that BAP + NAA are basically involved in the development of callus. Callusing started at the cut ends or along the entire surface after 8 days of culture and after 18 to 21 days the entire explant turned with a mass of green soft and friable callus. Similar observations were also made in *N. tabacum.*[18,19] Contrary to this, in *N. tabacum* required exogenous cytokinin, it was reported that callus was produced from the leaf and internodal explants.[20]

The leaf explants are cultured on to the medium supplemented with BAP and NAA and produced higher amount of callus and few shoots; the callus is very competent and friable in nature. The regeneration of shoot primordia on the callus was observed clearly. On the same medium containing BAP+NAA (4.44 µM + 1.14 µM) or when calli was transferred to fresh medium, 25.00 ± 0.09 shoots were induced per explant within 20-25 days, the shoots elongated and grew and developed many leaves. The shoot proliferation effects of BAP+NAA observed is in consonance with other reports.[21,22]

Many medicinally important secondary metabolites like alkaloids, flavones, coumarin, saponins, triterpenes etc. are identified through phytochemical analysis. This analysis provide a valuable information about the types of phytoconstituents present in the extracts, which helps to select a particular extract and for further investigation to isolating the active principle.[23] Preliminary phytochemicals screening of tobacco callus ethanol extract revealed the presence of alkaloids, steroids, tannins, phenol, glycorides, triterpenoids.

The functional constituents presencein the extract, identification of medicinal materials from the adulterate and evaluation of the qualities of medicinal materials identified through FTIR analysis.[24] In the present study the ethanol callus powder extract showed six peaks ranges between 3500 cm^{-1} and 450 cm^{-1} range. This peaks conformed the presence of alkyl halides, aliphatic amines, aromatic amines, nitro compounds, alkenes, alkyls respectively. This is in agreement with the study of[25] in *N. tabacum* seed biodiesel.

GCMS chromatogram analysis of ethanol callus extracts showed fourty seven peaks. Among these, eleven compounds are biologically active compounds. Pentadecanoic acid have antimicrobial activity[26], followed by n-Hexadecanoic acid withantioxidant, hypocholesterolemic nematicidal, antiandrogenic and pesticidal activity.[27] l-(+)-Ascorbic acid 2,6-dihexadecanoate showed significant antioxidant, antiinflammatory, antibacterial, analgesic, anti nociceptive properties.[28] Whereas Docosanic acid with 0.92 peak area has medicinal importance as an Detergents and floor polishes.[29] 1-Heptacosanol have been reported to have nematicidal , anticancer, antioxidant and antimicrobial activities.[30]Apartfrom this the compound Stigmasterol with 0.74 peak area with anti-tumor, cancer preventive and antiinflammatory activities.[31] The other compounds are may have pharmacheutical activity which is not known or not studied.

CONCLUSION

Our results clearly showed that the callus obtained from leaf explant of *N.tobacum* confirmed the presence of many bioactive compounds. It can be used for medicinal purpose and also a source of new drug formulation.

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