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IN VITRO INDUCTION OF CALLUS FROM DIFFERENT EXPLANTS OF *TERMINALIA ARJUNA* (ROXB.)WIGHT AND ARN.AND DETECTION OF ITS ACTIVE SECONDARY METABOLITES USING GC-MS ANALYSIS

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Abstract

Terminalis arjuna is one of the most important medicinal plants that used in folk medicine in different countries. The current study was achieved to identify the active phytochemical compounds in the callus using GC-MS technique. In order to induce callus, the leaves, petioles and internodes used as explants were taken from 10 years old trees and cultured in MS medium supplemented with different concentrations(0.1-4.0 $mg.l^{-1}$) of auxin 2, 4-D. Callus was extracted with hexane and analyzed with GC-MS for detection of its phytochemical components. The results displayed that internodal explants were the best for callus induction and proliferation under the concentration 3.0$mg.l^{-1}$ of 2, 4-D. The main compounds obtained from callus extract were Benzo[h] quinolone, 2, 4-dimethyl, 1H-Indole,5-methyl-2-phenyl-, 1, 2, 4-Oxadiazole,3- (1, 3-benzodioxol-5-yl) -5- [(4-iodo-1H-pyrazol-1-yl) methyl]-, alpha.-Amyrin; Urs-12-en-24-oic acid,3-oxo-, methyl ester, (+)-. This study focused on the detection of phytochemical active compounds *in vitro* from callus cultures and it is useful for the production of these compounds in the future in large quantities for pharmaceutical and commercial purposes.

Key words: *Terminalia arjuna*, Callus culture, Secondary metabolites, GC-MS analysis.

Introduction

The medicinal plants were used in folk medicine throughout the ages in many countries worldwide, because they contain various effective secondary metabolites of medicinal and pharmaceutical uses to treat different diseases and inflammations, as well as being safe in contrast to industrial drugs that carry many severe side effects (WHO, 1978). Therefore, and to overcome this problem, most of pharmaceutical companies tended to produce natural drugs by extracting them from different medicinal plants. Plants produce effective secondary metabolites such as alkaloids, phenols, flavonoids, terpenoids, tannins and quinones, which vary in type and production depending on plant type, age and environmental factors. These compounds protect plants against microbial infections, insects and animals (Okigbo and Oghonnaya, 2006). Because the acquisition of plant extracts from medicinal plants leads to the depletion of large numbers and large quantities of plant areas, so the plant tissue culture technique in recent years has provided different ways for obtaining effective secondary metabolites from different medicinal plant through callus and cell suspension cultures in small areas and over the year without relying on the season of plant growth or production of effective secondary metabolites, as well as, to propagate many species of plants in large numbers (Tiwari *et al.*, 2011; Sharma, 2012; Shanmuga *et al.*, 2015; Salim *et al.*, 2018).

Trees of *Terminalia arjuna* (Roxb.) Wight and Arn. are ornamental plants and have a very important medical value, belonging to genus *Terminalia* and family Combretaceae. They are commonly known as arjuna or arjun trees in English, Kumbuk in Sinhala, Neer Maruthu in Malayalam and Marudha Maram in Tamil. These plants grow in tropical and subtropical regions as native plants in India, Bangladesh and west Bengal (Orwa *et al.*, 2009; Biswas *et al.*, 2011). The *T. arjuna* tree's length about 20-25 meters with buttressed trunk, forming large canopy at the crown, and from which; the branches drop downwards. Leaves are conical in shape, green on the top and brown below, the bark is grey and smooth. Flowers are pale yellow which appear between March and June, fruits are fibrous woody (2.5-5cm) divided into

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five wings, appear between September and November (Biswas *et al.*, 2011). The medical benefit of *T. arjuna* is in the using of bark extracts to treat heart disease, which is why it got the name; guardian of heart, also, it is important in the treatment of hypertension, cancer, dysentery , ulcers and anti-microbial activity without any side effects (Dwivedi, 2007; Sharma, 2014; Gupta and Kumar, 2017).

T. arjuna plants are not found in Iraq, but rare numbers of seeds have been brought from Egypt and were cultured in small gardens, because of the lack of vitality of seeds and the difficulty of germination. Additionally, and due to the fact that the age and viability of seeds is very poor and difficult to germinate, some previous researches and studies have tended to use the technique of plant tissue cultu re in the micro propagation of these plants (Ramesh *et al.*, 2001). In the study of Arumugam and Gopinath (2011), they used different explants such as leaves, epicotyls, cotyledons and hypocotyls of *T. arjuna* seedlings for *in vitro* propagation of these trees. Based on this, and due to the medicinal importance of *T. arjuna* plant, the current study was performed to determine the superiority of any explant; leaf, leaf petiole or internode for the induction of callus and the evaluation of the active secondary metabolites in it using the technique of gas chromatography-mass spectrometry (GC-MS).

Material and Methods

Source of explants and callus induction conditions

The young leaves, leaf petioles and internodes (used as explants) were collected from eight years old trees of *Terminalia arjuna* (fig. l), and in the plant tissue culture laboratory; these explants were well washed with running tap water and liquid soap, then, they immersed in 70% ethanol for one minute inside the laminar-air flow cabinet, then they transferred to 0.l% solution of mercuric chloride ($HgCl_2$) for sterilization for five minutes followed by rinsing with sterile distilled water three times (two minutes for each one). After that, explants were cut into pieces with a length of 0.8-1.0 cm (leaves were cut into 0.8 cm diameter pieces using cork- borer). Explants were then cultured on MS medium (Murashige and Skoog, 1962) with full strength of its salts supplied with nicotinic acid ($1.0mg.l^{-1}$), pyridoxine-HCl ($0.5mg.l^{-1}$), thyamine-HCl ($1.0mg.l^{-1}$), glycine ($2.0mg.l^{-1}$), *myo*-inositol ($100mg.l^{-1}$), sucrose ($30g.l^{-1}$) agar-agar ($7.0\ g.l^{-1}$) and supplemented with 2, 4- Dichlorophenoxy acetic acid (2,4-D) at different concentrations (0.1, 0.5, 1.0, 2.0, 3.0 or 4.0 $mg.l^{-1}$). The pH of medium was adjusted to 5.7 using 0.1 N of sodium hydroxide or hydrochloric acid, then, the medium was placed on the hot-plate magnetic stirrer to melt the agar. After that, medium was poured into glass containers (2.5*15cm) with 20ml per one. Sterilization of medium was achieved using autoclave with 121ºC under pressure of 1 bar. Ten replicates were cultured for each treatment, and all cultures were incubated in growth room at 27ºC under light intensity of 1000lux for 16hrs. photoperiod. Callus induction frequency (%), fresh and dry weights of callus (mg) were calculated after 30 days of incubation to determine the best plant part and concentration of 2,4-D for callus induction, in order to continue of callus multiplication on it for the next experiment.

Fig. 1: *Terminalia arjuna* plant

Preparation of callus extract

After gaining of enough amounts of callus from previous experiment, they were dried in oven at 70ºC for 48hrs.and grinded to fine powder. Extraction was performed by adding 5ml of hexane to 5mg of powdered callus and kept for 6hrs., then, the extract was centrifuged for 10 minutes at 5000 rpm. Finally, the callus crude extract was collected in glass vials for further analysis in the experiment.

Detection of secondary metabolites in callus extract using GC-MS analysis

The callus crude extract of *T. arjuna* was analyzed using GC-MS (Agilent 19091S-33UI) apparatus equipped with National Institute of Standard and Technology (NIST) Library; column HP-5MS capillary column (cross bond 5% diphenyl-95% dimethylpolysiloxane); 30m× 250µm with a 0.25 µm film thickness; injection temperature, 290ºC; column temperature, 40ºC held to 2 min., rising 4ºC/min, then rising to 290ºC and held for 5 min.; injection mode, split; split at ratio 1:20; injected volume, 5 µl. The carrier gas was Helium (99.99%); acquisition mass range, 40-600m/z. The active compounds of the extract were

identified by comparing their retention indices with NIST library.

Statistical analysis

The experiments of callus inductions were analyzed using completely randomized design (CRD). The experiments were repeated two times and the means were assessed using the least significant differences (LSD) test at P=0.05 (SAS, 2004).

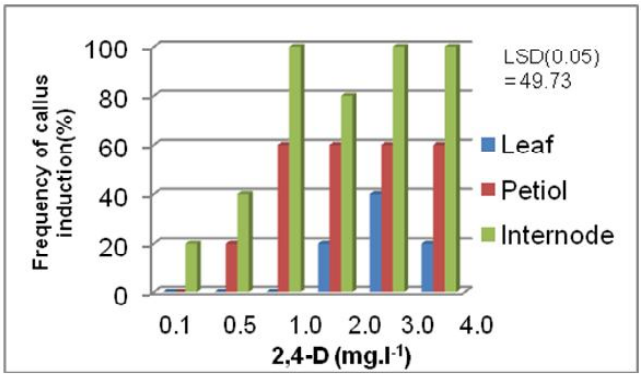
Results and Discussion

Effect of 2,4-D and explant type on callus induction

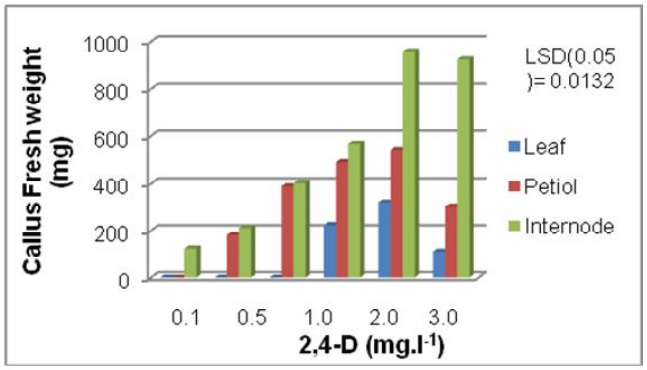
There were significant differences among the type of explants and 2, 4-D concentrations in the formation of callus (Fig. 2). Callus which was induced from all explants types was depending on plant growth regulators in MS medium but differences were found among different types of explants to form callus. Data in fig. 2a, showed the superiority of concentrations 1.0, 3.0 and 4.0 mg.l^{-1} of 2,4-D which gave rapid and highest *in vitro* response (100%) for callus induction from internodal explants than the leaves and petioles explants with 2, 4-D concentrations. This confirms that the concentration and kind of plant growth regulators and the kind of explants are the most determinant factors in the callus induction (George *et al.*, 2008).

The influence of explants and concentration of growth regulators on callus proliferation was evident in previous studies. Arumugan and Gopinath (2011) obtained a percentage of callus induction ranged between 40-95% from leaves of seedlings of *Terminalia arjuna* at age 10-12 days on MS and LS media as compared with hypocotyls, epicotyls and cotyledons. On the other hand, Dhaker *et al.* (2013) used kinetin with 2, 4-D to gain the best percentage of callus induction from leaves explants that were taken from 15 days old seedlings of *Terminalia bellerica*.

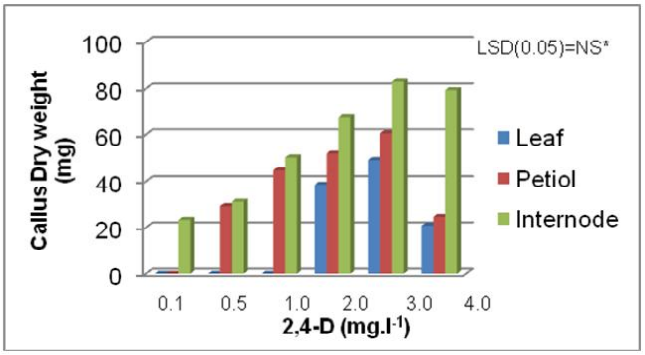
The results of fig. 2b, showed that the internodal explants significantly exceeded the leaves and petioles explants by giving the highest average of fresh weight of 958.6mg at concentration 3.0 mg.l^{-1} of 2, 4-D (used for multiplication of callus for next experiment) followed by the concentration 4.0 mg.l^{-1} with the same explants. This result is in agreement with Souza *et al.* (2014) who used different concentrations of 2, 4-D to stimulate and develop of callus that induced from seeds of *Boehaavia paniculata*. Whereas, there was no significant difference in the dry weight of the callus induced from different explants with all concentrations of 2, 4-D (fig. 2c). the callus induced from internodal explants was illustrated in fig. 3.



(a)



(b)



(c)

Fig. 2: Callus induction from different explants of *Terminalia arjuna*. (a) percentage of callus induction. (b) callus fresh weight(mg). (c) callus dry weight(mg). NS*: not statistically significant.

Detection of secondary metabolites in *T. arjuna* callus using GC-MS analysis

The gas chromatography mass spectrometry analysis of the hexanic extract of the *T. arjuna* callus (table 1 and fig, 4) indicated the presence of 43 bioactive compounds with different relative contents at different retention times. The main compounds observed were: benzo [h] quinolone, 2, 4-dimethyl- (14.370%); lH-Indole, 5-methyl- 2-phenyl- (11.377%); l, 2, 4-Oxadiazole, 3-(1,3-

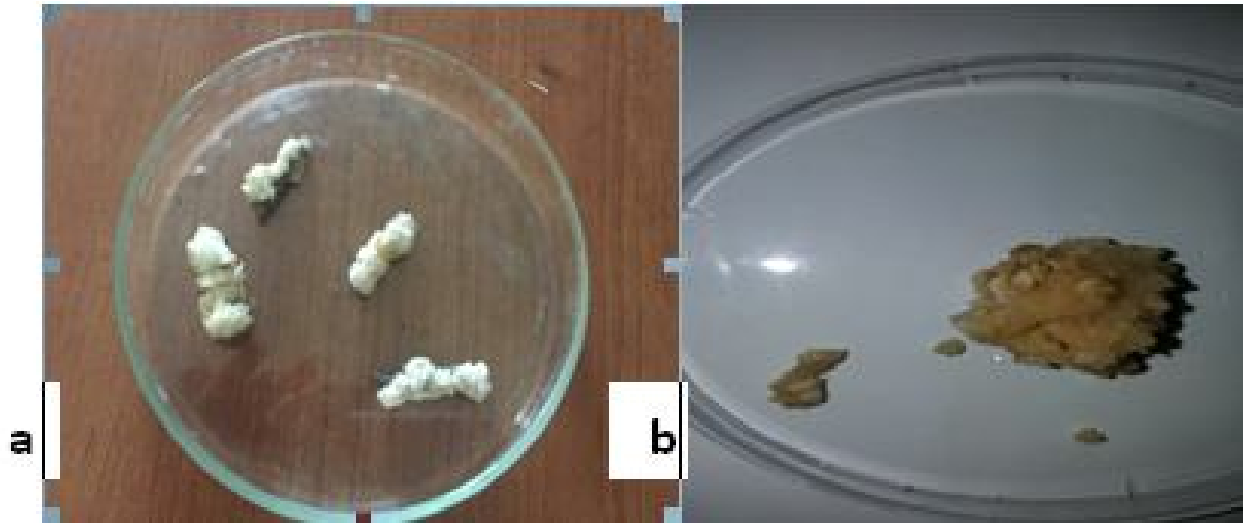


Fig. 3: Callus induction from *T. arjuna* internodal explants. (a) after 30 days. (b) multiplication of callus on MS medium enriched with 3.0mg.l^{-1} 2, 4-D, bar: 1cm.

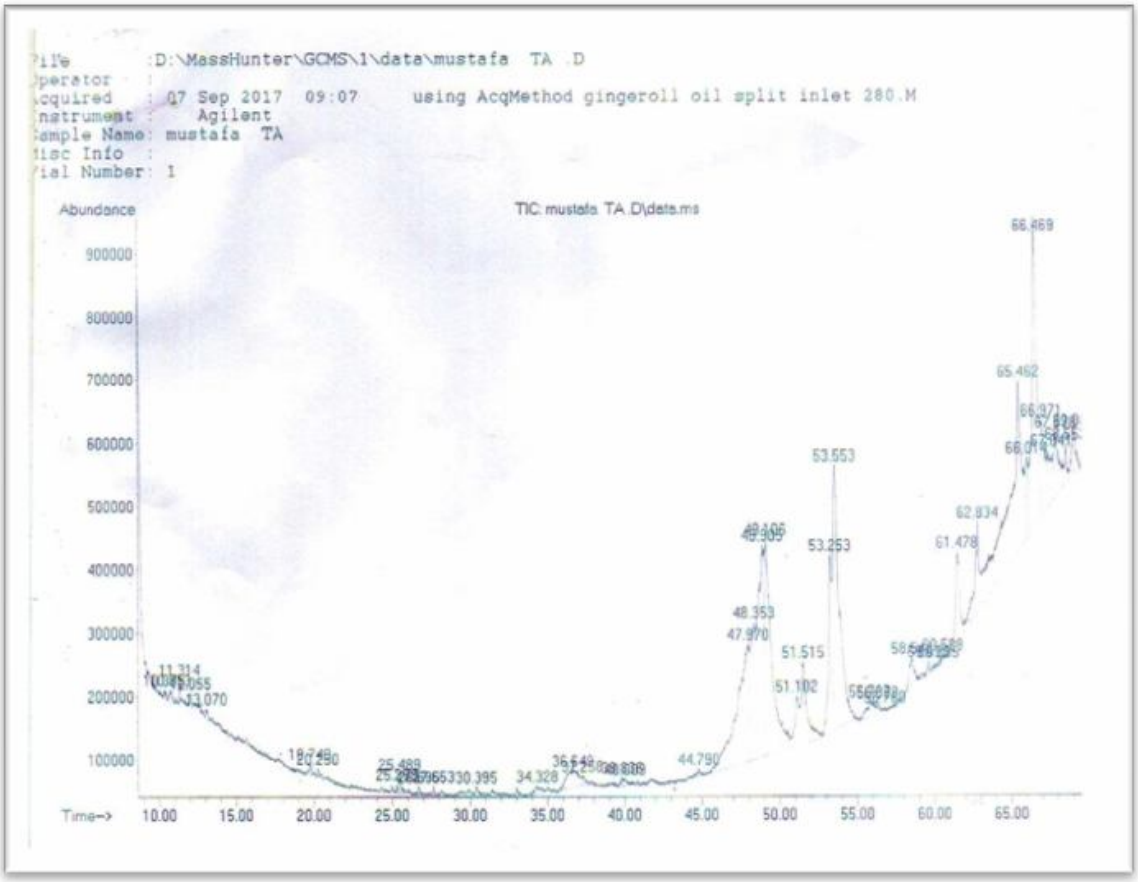


Fig. 4: The GC-MS chromatogram of *T. arjuna* callus extracted with hexane.

benzodioxol-5-yl)-5-[(4-iodo-lH-pyrazol-1-yl) methyl]- (10.297%);.alpha.-Amyrin (9.896%); Urs-12-en-24-oic acid, 3-oxo-,methyl ester, (+)- (9.868%);.beta-Amyrin trimethylsilyl ether(8.850%); Methadone N-oxide (4.291%);.alpha.-Amyrin, trimethylsilyl ether (4.198%); Azetidine, 1-benzyl-3,3-dimethyl-2-phenyl-(3.335%); 2-Ethylacridine (2.992%); Anthracene, 9, 10-dihydro-9, 9, 10-trimethyl-(2.725%); Pentacyclo [19.3.1.1(3,7).9,13) .1(15,19)] octacosa-1(25), 3, 5, 7(28), 9, 11, 13 (27), 15, 17, 19(26), 21, 23-dodecane-25, 26, 27, 28-tetrol, 5, 11, 17, 23-tetrakis (1,1-dimethylethyl) (2.656%) and Benzene, 2- [(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl- (2.195%). There were small percentages of other compounds, most of them:1-Methyl-3-phenylindole

Table1: Secondary metabolites of *Terminalia arjuna* callus analyzed with GC-MS

Peak . No	R.T.*	Compounds	Area	% of Total
1	10.302	Propane, 1-isocyanato-	1365477	0.129
2	10.712	1-Pentene, 2-methyl-	2217404	0.210
3	11.316	1-Pentene, 2-methyl-	981435	0.093
4	12.050	Pentane, 2,3-dimethyl-	1885915	0.178
5	13.075	1-Pentene, 2-methyl-	1303985	0.123
6	19.742	Propanenitrile, 3-(hexyloxy)-	2233691	0.211
7	20.249	2-Heptafluorobutyroxydodecane	727970	0.069
8	25.299	Androst-5-ene-3,17-diol, 4,4-dimethyl-, diacetate, (3.beta.,17.beta.)-	544959	0.052
9	25.493	Milamine, tris(trimethylsilyl) derivative	2623558	0.248
10	26.690	Benzothiazole, 2-[1-(2-phenylethyl)-2-benzimidazolylmethylthio]-	861851	0.082
11	27.650	Cycloheptasiloxane, tetradecamethyl-	1109868	0.105
12	30.391	Benzo[1,2-c:4,5-c']dipyrrole-1,3,5,7(2H,6H)-tetrone,2,6-bis(2-chlorophenyl)-	1108417	0.105
13	34.329	Hexanoic acid, 2-phenylethyl ester	2010937	0.190
14	36.648	Cyclobutane, 1,3-diphenyl-,trans-	15249482	1.443
15	37.252	[2.2] Paracyclophane	1637833	0.155
16	39.842	Sydnone, 4-acetyl-3-phenyl-	1701703	0.161
17	40.003	Pyrazino[1,2-a]indole,1,2,4,4,10,10a-hexahydro-4-phenyl-	1297673	0.123
18	44.794	Pyrene, hexadecahydro-	3264383	0.309
19	47.965	.alpha.-Amyrin	104568841	9.896
20	48.354	.alpha.-Amyrin, trimethylsilyl ether	44360079	4.198
21	48.904	.beta.-Amyrin trimethylsilyl ether	93517722	8.850
22	49.109	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	104272530	9.868
23	51.105	Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	14935533	1.413
24	51.515	Azetidine, 1-benzyl-3,3-dimethyl-2-phenyl-	35243156	3.335
25	53.252	Methadone N-oxide	45341683	4.291
26	53.554	1H-Indole, 5-methyl-2-phenyl-	120218387	11.377
27	55.787	(E)-2-bromobutyloxychalcone	8529601	0.807
28	56.305	Thiocarbamic acid, N,N-dimethyl, S-1,3-diphenyl-2-butenyl ester	2119719	0.201
29	56.780	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	682907	0.065
30	58.538	Cyclopenteno[4.3-b]tetrahydrofuran,3-[(4-methyl-5-oxo-3-phenylthio) tetrahydrofuran-2-yloxymethylene]-	11092593	1.050
31	59.596	Hexahydropyridine,1-methyl-4-[4,5-dihydroxyphenyl]-	3740869	0.354
32	60.232	2,4,6-Cycloheptatrien-1-one,3,5-bis-trimethylsilyl-	1561361	0.148
33	60.523	Propiophenone,2'-(trimethylsiloxy)-	1275422	0.121
34	61.473	Pentacyclo[19.3.1.1(3,7).1(9,13).1(15,19)]octacosa-1(25),3,5,7(28), 9,11, 13(27), 15, 17,19(26),21,23-dodecaene -25,26,27,28-tetrol,5,11,17,23-tetrakis(1,1-dimethylethyl)	28061114	2.656
35	62.832	2-Ethylacridine	31616353	2.992
36	65.465	Benzo[h]quinoline,2,4-dimethyl-	151836847	14.370
37	66.015	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	14508205	1.383
38	66.468	1,2,4-Oxadiazole,3-(1,3-benzodioxol-5-yl)-5-[(4-iodo-1H-pyrazol-1-yl)methyl]-	108800958	10.297
39	66.975	Anthracene,9,10-dihydro-9,9,10-trimethyl-	28793682	2.725
40	67.644	1-Methyl-3-phenylindole	17059688	1.615
41	67.881	Benzene,2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl-	23194340	2.195
42	68.550	Tris(tert-butyldimethylsiloxy)arsane	7242464	0.685
43	69.079	Trimethyl[4-(2-methyl-4-oxo-2-pentyl)phenoxy]silane	11948742	1.131
Total			**1056649341**	**99.999**

R.T. : Retention time(minute)

(1.615%); Cyclobutane,1, 3-diphenyl-, trans-(1.443%); Phenol, 2, 2'-methylenebis [6-(1,1-dimethylethyl)-4-methyl- (1.413%); 1, 4-Benzendiol, 2, 5-bis (1,1-dimethylethyl) -(1.383%); Trimethyl [4-(2-methyl-4-oxo-2-pentyl) phenoxy] silane (1.131%) and Cyclopenteno [4.3-b] tetrahydrofuran,3-[(4-methyl-5-oxo-3-phenylthio) tetrahydrofuran -2-yloxymethylethylene] -(1.050%). This means the efficacy of the present method for extracting and detecting the bioactive compounds from callus of *T. arjuna*. Whereas, in the study of Gupta and Kumar (2017), they obtained 21compounds when they extracted the leaves of *T. arjuna* with chloroform: methanol method.

Pharmaceutical and biological activities of the secondary metabolites detected in *T. arjuna* callus using GC-MS analysis

In this study, most of the detected secondary metabolites or phytochemical compounds in the callus extract of *T. arjuna* have valuable and important pharmacological and biological activities (table-2). Among these compounds, there were twenty one compounds have possessed antimicrobial, anti-inflammatory, antioxidant and anticancer properties, namely: Propanenitrile,3-(hexyloxy)-; 2-Heptafluoro-butyroxy-dodecane; Benzothiozole,2-[1-(2-phenylethyl)-2-benzimidazolylmethylthio]-; Cycloheptasiloxane, tetradecamethyl-; Benzo[1, 2-c:4, 5-c'] dipyrrole-1, 3, 5, 7 (2H, 6H) -tetrone, 2, 6-bis (2-chlorophenyl)-, Sydnone, 4-acetyl-3-phenyl-; Phenyl, 2, 2'-methylenebis [6-(1, 1-dimethylethyl) - 4-methyl-; lH-Indole, 5-methyl-2-phenyl-(E) -2-bromobutyloxychalcone; Octasiloxane, 1, 1, 3, 3, 5, 5,7 , 7, 9, 9, 11, 11, 13, 13, 15, 15-hexadecamethyl-; Hexahydro- pyridine-1-methyl-4-[4, 5-dihydroxyphenyl]-Propiophenone, 2'-(trimethylsiloxy)-; Pentacyclo [19.3.1.1(3, 7).1(15, 19)] octacosa-1(25), 3, 5, 7(28), 9, 11, 13(27), 15,17,19(26),21,23-dodecaene-25,26,27,28-tetrol,5,11,17,23-tetrakis(1,1-dimethylethyl); 2-Ethylacridine; Benzo[h] quinolone, 2, 4-dimethyl-; 1, 4-Benzenediol, 2, 5-bis (1, 1-dimethylethyl)-; 1, 2, 4-Oxadiazole, 3-(1, 3-benzodioxol-5-yl)-5-[(4-iodo-lH-pyrazol-1-yl) methyl]-; Anthracene, 9, 10-dihydro-9, 9, 10 trimethyl-; Benzene,2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl-; Tris (tert-butyldimethylsiloxy) arsane; Trimethyl [4-(2-methyl-4-oxo-2-pentyl) phenoxy] silane and Thiocarbamic acid, N, N-dimethyl, S-1, 3-diphenyl-2-butenyl ester (Celis *et al.*, 2011; Hou *et al.*, 2011; Arya *et al.*, 2012; Patil and Rathod, 2014; Yadav *et al.*, 2016; Peng *et al.*, 2017; Shaik and Mokat, 2017).

On the other hand, the most important compound. alpha.-Amyrin and its derivatives were reported to have properties of cancer preventers, anti-ulcer effect, hepatitis, wound and burns treatment, antidiabetic, antiviral infections, antimicrobial and anti-inflammations (Hernandez-Vazquez *et al.*, 2012; Okoye *et al.*, 2014).

The other compounds that were found in the callus extract and carry pharmaceutical properties were: Androst-5-ene-3, 17-diol,4, 4-dimethyl-, diacetate, (3.beta.,17.beta.)-, which a natural precursor of testosterone and other androgens and estrogens, whereas, cyclobutane, 1, 3-diphenyl ester exhibits estrogenic activity(Miller *et al.*, 2013). Melamine, tris (trimethylsilyl) derivative which is a nitrogen fertilizer of crops, part core of some drugs including Lamartine and Alteramine (Barrett and Gilbert, 2006). Hexanoic acid which is a fatty acid that is used in the manufacture of flavors, vanilla and drugs (Uddin *et al.*, 2013). Pyrazino [1, 2-a] indole, 1, 2, 4, 4, 10, 10a-hexahydro-4-phenyl- and its derivatives were recorded as antiproliferative agents against human leukemia K562 cells(Romagnoli *et al.*, 2010). Moreover, other compounds that have been mentioned with different pharmaceutical and biological properties such as: Urs-12-en-24-oic acid,3-oxo-,methyl ester,(+)- used as antiasthema, diuretic, antiarthritic, anti-inflammatory and as a precursor for synthesis of other amyrin forms(Vidhya and Udayakumar, 2015); Azitidine,1-benzyl-3,3-dimethyl-2-phenyl- was recorded as antihyperglycemic, vasopressin vla antagonist, anticancer, antitubercular and anti-inflammatory (Gandhi *et al.*, 2017); Methadone N-oxide used to treat pain, neuropathic pain and opioid detoxification (Dinis-Oliveira, 2016); Cyclopenteno [4,3-b] tetrahydrofuran, 3-[(4-methyl-5-oxo-3-phenylthio)tetrahydrofuran-2-yloxymethylene]-was an intermediate precursor of ascorbic acid synthesis (Duke, 2015). Other compounds had different activities like: Propane, 1-isocyanato- used for *in vitro* inhibition of alcohol dehydrogenase; 1-Pentene, 2-methyl- used in drugs manufacture; Pentane, 2, 3-dimethyl- as alkylation agent and 1-Methyl-3-phenylindole used in colorimetric assay of lipid peroxidation. While, no pharmaceutical and biological activity was recorded for the compound 2, 4, 6-Cycloheptatrine-1-one, 3, 5-bis-trimethylsilyl-.

The current study is the first scientific record in Iraq to detect the bioactive secondary metabolites of the *in vitro* induced callus from *Terminalia arjuna* internodes using GC-MS analysis. The medicinal and biological importance of *T. arjuna* is attributed to its containment of so many effective phytochemical compounds that exhibit many antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, anticancer and anti-ulcer activities. This is important to benefit from these important

Table2: Pharmaceutical and Biological activities of the secondary metabolites of *Terminalia arjuna* callus analyzed with GC-MS

Name of Compound	Pharmaceutical or Biological activity
Propane, 1-isocyanato-	*In vitro* inhibition of alcohol dehydrogenase
1-Pentene, 2-methyl-	Precursors in drugs manufacture
Pentane, 2,3-dimethyl-	Alkylation agent
Propanenitrile, 3-(hexyloxy)-	Antimicrobia1, antioxidant activities
2-Heptafluorobutyroxydodecane	Antimicrobia1activity
Androst-5-ene-3,17-diol, 4,4-dimethyl-, diacetate, (3.beta., 17.beta.)-	A precursor of testosterone and other androgens and estrogens synthesis, antitumor
Milamine, tris(trimethylsilyl) derivative	A nitrogen fertilizer of crops, part core of some drugs including Lamartine, and alter amine
Benzothiazole, 2-[1-(2-phenylethyl)-2-benzimidazoly--lmethylthio]	Anti-diabetic, anticonvulsant, ant tubercular, anti-microbia1
Cycloheptasiloxane, tetradecamethyl-	Anti-microbia1, anticancer, anti-oxidant, fragrance, skin conditioning
Benzo[1,2-c:4,5-c']dipyrrole-1,3,5,7(2H,6H)-tetrone, 2,6-bis(2-chlorophenyl)-	Anti-microbia1, Fungicides,
Hexanoic acid, 2-phenylethyl ester	As a component of vanilla, flavors and drugs manufacture
Cyclobutane, 1,3-diphenyl-, trans-	Exhibit estrogenic activity
[2.2]Paracyclophane	Catalysts for enantioselective addition in situ-prepared Alkynylzinc reagents
Sydnone, 4-acetyl-3-phenyl-	Anticancer, anti-diabetic, antimicroboa1, antioxidant,
	anti-inflammatory
Pyrazino[1,2-a]indole,1,2,4,4,10,10a-hexahydro-4-phenyl-	Anti-proliferative agent against human leukemia K562 cells
Pyrene, hexadecahydro-	Dyes and p1esticers precursor
alpha.-Amyrin	Antihepatitis,anti-microbia1, anti-diabetic, anti-funga1, anti-vira1-infections, hypolipidemic action, sedative effect, anticancer, anti-ulcer effect and anti-infl ammations
alpha.-Amyrin, trimethylsilyl ether	Anti-microbia1, anticancer, anti-funga1, anti-diabetic
beta.-Amyrin trimethylsilyl ether	Anti-microbia1, anticancer, anti-funga1, anti-diabetic, antioxidant
Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	Anti-arthritic, diuretic, and anti-asthma, precursor for synthesis of other amyrin forms
Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	Antibacteria1against pathogenic bacteria
Azetidine, 1-benzyl-3,3-dimethyl-2-phenyl-	Antihyperglycemic, vasopressin v1a antagonist, anticancer, Antitubercular, anti-infl ammatory
Methadone N-oxide	Treatment of pain, neuropathic pain, opioid detoxification
1H-Indole, 5-methyl-2-phenyl-	Anti-inflammatory, analgesic activity
(E)-2-bromobutyloxychalcone	Antioxidant, anti-inflammatory
Thiocarbamic acid, N,N-dimethyl,S-1,3-diphenyl-2-butenyl ester	Anticancer activity
Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexade-camethyl	Anti-bacteria1activity
Cyclopenteno[4.3-b]tetrahydrofuran,3-[(4-methyl-5-oxo-3-phenylthio) Tetrahydrofuran-2-yloxymethylene]-	Intermediate precursor of ascorbic acid (vitamin C) synthesis
Hexahydropyridine,1-methyl-4-[4,5-dihydroxyphenyl]-	Antioxidant activity
2,4,6-Cycloheptatrine-1-one,3,5-bis-trimethylsilyl-	No biological activity was reported
Propiophenone,2'-(trimethylsiloxy)-	Antifungal, anti-leshmanial, insecticidal activity
Pentacyclo[19.3.1.1(3,7).1(9,13).1(15,19)]octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaene-25,26,27,28-tetrol,5,11,17, 23-tetrakis(1,1-dimethylethyl)	Antiviral activity

Table 2 continude .....

Table 2 continude .....

Name of Compound	Pharmaceutical or Biological activity
2-Ethylacridine	Antitumor, antioxidant
Benzo[h]quinoline,2,4-dimethyl-	Anticancer, antibacteria1, antifunga1, antimalarial effects, mutagenic activities in strain TA100of *Salmonella*
1,4-Benzenediol,2,5-bis(1,1-dimethylethyl)-	Uses in perfumery, antioxidant, anti-mycobacterial
1,2,4-Oxadiazole,3-(1,3-benzodioxol-5-yl)-5-[(4-iodo-1H-pyrazol-1-yl)methyl]-	Anticancer, anti-diabetic, anti-inflammatory
Anthracene,9,10-dihydro-9,9,10-trimethyl-	Anticancer, antibacteria1, anti-inflammatory
1-Methyl-3-phenylindole	Used in colorimetric assay of lipid peroxidation
Benzene,2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4 -methyl-	Antibacteria1 activity
Tris(tert-butyldimethylsilyloxy)arsane	Antioxidant, antibacteria1, antifunga1
Trimethyl[4-(2-methyl-4-oxo-2-pentyl)phenoxy]silane	Antioxidant, antibacteria1, anti-inflammatory

compounds at the pharmaceutical level. As there are many pathogenic microorganisms resistant to industrial treatments, so most health and educational institutions worldwide have tended to benefit from medicinal plants and extract natural therapeutic drugs from them (Elangovan *et al.*, 2015; Gupta and Kumar, 2017).

Conclusion

The current study is useful in the detection of several active phytochemical compounds in the callus of *T. arjuna* by extracting with hexane, allowing the opportunity in the future to produce these active compounds in large quantities pharmaceutically and commercially for the manufacture of drugs, cosmetic and skin care products using plant tissue culture technology within small areas and without damage or drain large areas of cultivated plants.

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