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Gas Chromatography-Mass spectrometry Analysis of Phytocomponents Present in *Pimpinella anisum* L. Callus Cultures as Affected by Yeast and Phenylalanine Application

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Abstract

Long times ago, anise (Pimpinella anisum L.) plant, which belonging to umbelliferae family, was used in Egyptian folk medicine. In the current study, the effects of yeast extract as an elicitor and phenylalanine as a precursor on the accumulation of bioactive constituents in anise callus cultures were documented by gas chromatography-mass spectrometry (GC-MS) techniques. It is revealed that P. anisum callus extracts contain different biologically active compounds with antioxidant, antimicrobial and anticancer potential. Cytotoxicity of anise callus extracts was studied to illuminate the vital assignment of many active constituents in diminution of the breast cancer growth and also their antitumor activity.

Key words: Pimpinella anisum; Medicinal plants; GC-MS; Active constituents.

1. Introduction

From ancient times, plant products are known as a prosperous source for heeling effect with varied phytocomponents and bioactivities allied with many heath disorders including cancer [1]. In recent times, the uses of folk medicinal plants as complementary and alternative medicine were considerably increased along with their use as flavor additives [2,3]. At present there is rising research efforts regarding the dynamic function of plant products [4]. Extracts screening of folk medicinal plants for the active components to replacing synthetic drugs are a challenge [5, 6].

Anise (*Pimpinella anisum* L.), is an annual medicinal plant belonging to Apiaceae family and native to Egypt. Medicinal plants contain substances that could be used for curative purposes [7]. In recent times, this flavor plant has drawn more consideration because of the antioxidant, antimicrobial and anticancer affects on human health [8]. Moreover, p. anisum was used in pharmaceutics, perfumery, food and cosmetic industries [9].

Different *Pimpinella* species has been investigated for their essential oil composition [11, 12, 13, 14, 15, 17]. The major components were widely varied within this species: Embong *et al*. [10] established (E)-anethole (72.2%), (Z)-anethole (1.1%), anisyl ceton (0.9%), β-caryophyllene (0.8%) and carvone (0.3%); [13] identified twenty-two components of the anise oil with the major components of (E)-anethole (85%), (Z)-anethole (2.2%) and methyl chavicol (1.02%); Askari *et al*. [16] identified (E)-anethole (90%), eugenyl acetate (2%), γ-gurjunene (1.85%) and estragole (1.04%).

The yield and quality of anise are considerably affected by many factors including climatic conditions, genetic potential of the cultivar and agronomic practices [16]. Diseases of anise are commonly fungal in nature like Alternaria blight, downy mildew, powdery mildew and rust disease. Tissue culture is an alternative tool to avoid such cultivation problems and hence raise the outcome benefits from anise culture [17].

The accumulation of plant secondary metabolites is a common response of plants to biotic and abiotic stresses. Consequently, elicitation, treatment is one of the most effective strategies for improving secondary metabolite production in plant tissue cultures [18,19].

Phenylalanine is an amino acid, the precursor of the phenylpropanoide pathway leading to the formation of phenolic acids, flavonoids and other phenolic compounds. Phenylalanine has been effectively used *in vitro* for metabolite production in numerous plant cultures [20].

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Many researchers were confirmed the application of yeast extract as one of the biotic elicitors. Yeast extracts stimulated ethylene biosynthesis in tomato, bacterial resistance in *Phaseolus vulgaris* and also elicited the production of tanshinone in the root culture of *Perovskia abrotanoides* [21].

Acetone is a potent solvent that dissolves and extracts with ease, and its high evaporation rate expedites the evaporation portion of the extraction process, sometimes eliminating the need to use heat Acetone solvent seems to be favored in extraction of bioactive constituents with antitumor activity [22, 23].

To the best of our knowledge, no studies have been documented on the effects of yeast and phenylalanine on the active constituents of *P. anisum* cell cultures. Herein for the first time, the composition of the volatiles from the acetone extracts of callus cultures grown on different concentrations of yeast and phenylalanine was reported.

The leading objective of the current study was focused on identification and quantification of the possible bioactive components found in calli cultures of *Pimpinella anisum* L. by gas chromatography-mass spectrometry instrument to attain novel compounds for therapeutic industry.

Material and Methods

Plant material and tissue culture experiment

Seeds of anise were obtained from the Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt. The tissue culture experiment and culture conditions were reports elsewhere [24].

Precursor and elicitor preparation and administration

This research was conducted to appraise the effect of a precursor and biotic elicitor, each at different concentrations on callus growth and active ingredient content. Pieces of callus about (0.5 g) were cultured on MS medium [25] supplemented with 1 mg/l NAA + 1 mg/l 2, 4-D + 2 mg/l KIN. Precursor and elicitor preparation were done by dissolving the required amount into the sterile distilled water; filter sterilized and added before solidification of the autoclaved media. Each of elicitor and precursor were added to the *Pimpinella anisum* callus cultures in triplicate. Culture medium without elicitor and precursor were also included as control set.

Yeast elicitor

Ten grams of yeast extract was dissolved in 100 ml of double distilled water (10 g/100 ml) and then ethanol was added up to 80% (v/v) and was kept at 4 °C for 3 days for precipitation. The supernatant was decanted and the precipitate was re-dissolved in 100 ml double distilled water then autoclaved and was used as elicitor [26].Tested concentrations were 0; 1.82; 3.64 and 5.46 mM/l. free yeast medium was used as control medium.

Phenylalanine precursor

A stock solution of 10 mM concentration was prepared by dissolving phenylalanine in distilled water, sterilized using 0.2µm micro filters and concentrations of 0, 2, 4 and 6 mM/l were prepared. Sterile filtered concentrations of Phenylalanine were added before solidification of the autoclaved media. Free Phenylalanine medium was used as control medium.

Extraction

In vitro shoot tip derived calli of *Pimpinella anisum* L. (20 g) which grown on control MS-medium (elicitor free) and MS-medium fortified with different concentrations of either phenylalanine (2, 4, 6 mM/l) or yeast extract (1.82, 3.64, 5.46 mM/l) were extracted separately using a soxhlet apparatus with 250 ml acetone solvent for 3 hours. Crude extracts for each treatment were filtered using 0.45 µm filter before subjected to centrifugation at 12 000 rpm three times. The obtained supernatants were evaporated and concentrated by vacuum distillation using rotary evaporator device (Buchi, USA, R-210/215; Switzerland). The extracts were separated from the solvent using a rotary evaporator at 30–40°C and stored at 4 °C for GC-MS analysis and biological studies.

Gas chromatographic-mass spectrometric

The extracts were analyzed by GC-MS to determine the total content of volatile constituents and other phytocomponents along with their antitumor activity. The analysis was performed using a Thermo Scientific, USA, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS detection The ionization voltage was 70 eV, mass range m/z 39-400 a.m.u. The quantification of all the identified phytocomponents and volatile constituents was investigated using a percent relative peak area. The identity of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library NIST [27], WILEY [28] and also with published literatures.

Cytotoxicity of callus culture extracts:

Cytotoxicity of anise extracts derived from shoot tip calli was examined on breast cancer cell line (MCF7), at the National Cancer Institute, Cairo University Egypt, by SRB assay as described by Suresh et al. [29] using color intensity measurements by ELISA RADER at a wavelength of 570 nm. The main values of results were calculated as follows: survival fraction=OD (treated cells)/OD (control cells), and IC50 value=the concentration of tested

extract required to produce 50% inhibition of cell growth, which was calculated using sigmoidal dose response curve fitting mode.

Results and Discussions

The cultural conditions for *Pimpinella anisum* L. callus initiation and development were achieved [24]**.** Our results revealed that the highest percentage of callus production was attained from shoot tip explants cultured on MS medium addend with 1 mg/l NAA + 1 mg/l 2, 4-D + 2 mg/l KIN**.** In the present study, acetone extract of anise (*Pimpinella anisum* L.) was analyzed through GC-MS.

The effect of 2mM phenylalanine on P. anisum callus cultures (Table 1), revealed the presence of 20 different phytocompounds; 2-Allyl-5-t-butylhydro quinone was found in maximum amount (17.38%) followed by 5-Octadecene,(E) (10.53%);1,2-Diphenyl-5-(t-butyl)acephenanthrylene (9.42%);1-Hexadecanol (8.84%);1-Docosene (8.31%) and Cyclohexane carboxylic acid, 1-phenyl, methyl ester (6.01%). The biological activity of these compounds was explained in table 7.

TABLE 1. Chemical composition (%) in essential oil analyzed by GC-MS for *Pimpinella anisum in vitro* calli grown on medium elicited by 2 mM/l L.phenylalanine

Compound Name	**Retention Time (RT)**	**Molecular Weight**	**Molecular Formula**	**Area%**
Dodecane,2,6,11-trimethyl	16.32	212	$C_{15}H_{32}$	2.26
Cyclotetradecane	17.44	196	$C_{14}H_{28}$	4.88
2-Allyl-5-t-butylhydroquinone	19.46	206	$C_{13}H_{18}O_2$	17.38
Hexadecane,2,6,11,15-tetramethyl (Eicosane)	19.77	282	$C_{20}H_{42}$	2.44
1-Hexadecanol	20.55	242	$C_{16}H_{34}O$	8.84
Docosane, 11-decyl	22.21	450	$C_{32}H_{66}$	1.86
Heneicosane	22.81	296	$C_{21}H_{44}$	3.05
Cyclobutane,1,2-diphenyl	23.04	208	$C_{16}H_{16}$	4.11
5-Octadecene,(E)	23.35	252	$C_{18}H_{36}$	10.53
7,9-Di-tert-butyl-1-oxas piro(4,5)deca-6,9-diene-2,8-dione	25.12	276	$C_{17}H_{24}O_3$	1.82
Heptacosane	25.54	380	$C_{27}H_{56}$	2.48
1-Docosene	25.87	308	$C_{22}H_{44}$	8.31
18-Ethyl-7,16-dimethyl 2,19-methano-18-azap entacyclo[10.6.0.0(3,8).0(3,11).1(12,16)]nonad ec-6-en-10-ol	27.71	343	$C_{22}H_{33}NO_2$	2.59
Behenic alcohol	28.19	326	$C_{22}H_{46}O$	4.42
1,2-Diphenyl-5-(t-butyl)acephenanthrylene	30.25	410	$C_{32}H_{26}$	9.42
Cyclotetracosane	30.56	336	$C_{24}H_{48}$	1.96
Cyclohexane carboxylic acid, 1-phenyl, methyl ester	31.36	218	$C_{14}H_{18}O_2$	6.01
Thiocarbamic acid,N,Ndimethyl,S-1,3-diphenyl-2-butenyl ester	32.33	311	$C_{19}H_{21}NOS$	1.77
2,3-Diphenylcyclopropyl)methyl phenylsulfoxide, trans	32.46	332	$C_{22}H_{20}OS$	3.43
Benzoic acid,3,5-dicyclohexyl-4-hydroxy,methyl ester	35.99	316	$C_{20}H_{28}O_3$	2.45

TABLE 2. Chemical composition (%) in essential oil analyzed by GC-MS for *Pimpinella anisum in vitro* calli grown on medium elicited by 4 mM/l L.phenylalanine

Compound Name	**Retention Time (RT)**	**Molecular Weight**	**Molecular Formula**	**Area%**
Ethane, 1,2diethoxy	1.71	118	$C_6H_{14}O_2$	24.56
1,2BIS(trimethylsilyoxy-2-(3'trimethylsilyoxyphenyl) Ethanone	2.19	384	$C_{17}H_{32}O_4Si_3$	1.66
Dimethyl 1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate	2.31	414	$C_{11}H_8Cl_6O_4$	1.09
Canthaxanthin	2.58	564	$C_{40}H_{52}O_2$	4.49
Methane,oxybis[dichloro	2.92	182	$C_2H_2Cl_4O$	10.86
1,5,9,13-Tetrathia-3,11-cyclohexadecaediol	3.09	328	$C_{12}H_{24}O_2S_4$	1.01
N,N-Dimethyl-2-Hpyran-2-iminium chloride	3.18	159	$C_7H_{10}ClNO$	1.61
dichloromethyl ethylsulfone	3.61	176	$C_3H_6Cl_2O_2S$	0.57
1-Docosene	25.80	308	$C_{22}H_{44}$	1.52
Carvone	34.67	150	$C_{10}H_{14}O$	14.88
ButylatedHydroxytoluene	50.97	220	$C_{15}H_{24}O$	21.82
Cetene	66.59	224	$C_{16}H_{32}$	1.24
1-Docosanol(CAS)	85.38	326	$C_{22}H_{46}O$	2.13
Rotenalone	92.58	410	$C_{23}H_{22}O_7$	6.59
Cyclotetracosane	93.68	336	$C_{24}H_{48}$	1.43
1,2-Benzenedicarboxylic acid, bis(2ethylhexyl)ester(CAS)	99.70	390	$C_{24}H_{38}O_4$	1.88
17-Pentatriacontene(CAS)	101.35	490	$C_{35}H_{70}$	0.82
Dimethoxy glycerol docosyl ether	105.19	460	$C_{27}H_{56}O_5$	0.51
Tetratetracontane	108.67	618	$C_{44}H_9O$	0.34
Methylsulfinato[2,3,7,8,12,13,17,18-octaethyl porphyrinato]indium	124.27	726	$C_{37}H_{47}InN_4O_2S$	1.00

Table (2) shows the effect 4mM phenylalanine on P. anisum calli cultures. The major constituents were Ethane, 1,2diethoxy (24.56%) followed by ButylatedHydroxytoluene (21.82%) and Carvone (14.88%).

Table (3) shows the effect 6mM phenylalanine on *P. anisum* calli cultures. 1-Octadecanol(CAS) was presented in highest amount (15.66%) followed by 1-Docosene (13.08%) and 1,2-Diphenyl-5-(t-butyl)acephenanthrylene (12.87%). The biological activity of these compounds was explained in table 7.

Table (4) shows the effect 1.82 mM yeast extract on *P. anisum* callus cultures. 1-Docosene was presented in highest amount (17.40%) followed by 5-Octadecene,(E) (17.02%) and 1,2-Diphenyl-5-(t-butyl)acephenanthrylene (9.20%). The biological activity of these compounds was explained in table 7.

Table (5) shows the effect 3.64 mM yeast extract on *P. anisum* callus cultures. 5-Octadecene,(E) was presented in highest amount (10.44%) followed by 1,2-Diphenyl-5-(t-butyl)acephenanthrylene (9.89%) and 1-Hexadecanol (9.43%). The biological activity of these compounds was explained in table 7.

TABLE 3. Chemical composition (%) in essential oil analyzed by GC-MS for *Pimpinella anisum in vitro* calli grown on medium elicited by 6mM/l L.phenylalanine

Compound Name	**Retention Time (RT)**	**Molecular Weight**	**Molecular Formula**	**Area %**
1-Heptadecene	15.99	238	$C_{17}H_{34}$	1.71
3-EICOSENE,(E)	16.13	280	$C_{20}H_{40}$	1.82
1-Octanol,2-butyl	16.28	186	$C_{12}H_{26}O$	2.40
Pentacosane (CAS)	19.07	352	$C_{25}H_{52}$	1.58
Phenol,2,4-bis(1,1-dimethylethyl)	19.46	206	$C_{14}H_{22}O$	14.50
Hexadecane, 2,6,11,15-tetramethyl (Ecosane)	19.77	282	$C_{20}H_{42}$	2.23
1-Hexadecanol	20.54	242	$C_{16}H_{34}O$	7.13
Nonadecane	20.63	268	$C_{19}H_{40}$	1.54
Heneicosane (CAS)	22.21	296	$C_{21}H_{44}$	1.88
Dodecane,2,6,11-trimethyl	22.80	212	$C_{15}H_{32}$	3.53
1-Decanol,2-hexyl	23.00	242	$C_{16}H_{34}O$	2.18
1-Octadecanol(CAS)	23.36	270	$C_{18}H_{38}O$	15.66
Octadecane	23.42	254	$C_{18}H_{38}$	2.13
7,9Ditertbutyl1oxas piro(4,5)deca-6,9-diene2,8 dione	25.11	276	$C_{17}H_{24}O_3$	1.72
Tricosane (CAS)	25.53	324	$C_{23}H_{48}$	2.52
1-Docosene	25.88	308	$C_{22}H_{44}$	13.08
Kaempferoltris(methyl ether)	27.72	328	$C_{18}H_{16}O_6$	2.48
Behenic alcohol	28.19	326	$C_{22}H_{46}O$	6.24
1,2-Diphenyl-5-(t-butyl)acephenanthrylene	30.25	410	$C_{32}H_{26}$	12.87
Cyclotetracosane	30.56	336	$C_{24}H_{48}$	2.81

TABLE 4. Chemical composition (%) in essential oil analyzed by GC-MS for *Pimpinella anisum in vitro* calli grown on medium elicited by 1.82 mM/l yeast

Compound Name	**Retention Time (RT)**	**Molecular Weight**	**Molecular Formula**	**Area%**
1-Decanol,2-hexyl	16.28	242	$C_{16}H_{34}O$	1.41
7-Tetradecene,(E)	17.44	196	$C_{14}H_{28}$	2.08
Dodecane,2,6,11-trimethyl	19.07	212	$C_{15}H_{32}$	1.16
2-tert-Butyl-4-isopropyl5-methylphenol	19.43	206	$C_{14}H_{22}O$	11.22
Nonadecane	19.76	268	$C_{19}H_{40}$	1.89
1-Hexadecanol	20.53	242	$C_{16}H_{34}O$	8.18
Hexadecane	20.63	226	$C_{16}H_{34}$	1.69
Pentacosane (CAS)	22.20	352	$C_{25}H_{52}$	1.57
Heptadecane,2,6,10,15-tetramethyl	22.79	296	$C_{21}H_{44}$	3.00
Trichloroacetic acid, pentadecyl ester	22.99	372	$C_{17}H_{31}Cl_3O_2$	1.92
5-Octadecene,(E)	23.34	252	$C_{18}H_{36}$	17.02
Octadecane	23.41	254	$C_{18}H_{38}$	2.01
7,9-Ditertbutyl-1-oxas piro(4,5)deca-6,9-diene 2,8-dione	25.10	276	$C_{17}H_{24}O_3$	1.98
Heneicosane,11(1-ethylpropyl)	25.52	366	$C_{26}H_{54}$	1.77
1-Docosene	25.87	308	$C_{22}H_{44}$	17.40
18-Ethyl-7,16-dimethyl 2,19-methano1-8-azap entacyclo[10.6.0.0(3,8).0(3,11).1(12,16)] nonadec-6-en-10-ol	27.68	343	$C_{22}H_{33}NO_2$	3.32
Behenic alcohol	28.17	326	$C_{22}H_{46}O$	7.90
1,2-Diphenyl-5-(t-butyl)acephenanthrylene	30.24	410	$C_{32}H_{26}$	9.20
n-Tetracosanol1	30.54	354	$C_{24}H_{50}O$	2.98
Cyclohexanecarboxylic acid, 1-phenyl, methyl ester	31.35	218	$C_{14}H_{18}O_2$	2.31

Table (6) shows the effect 5.46 mM yeast extract on *P. anisum* callus cultures.1,2-Diphenyl-5-(t-butyl)acephenanthrylene was presented in highest amount (15.96%) followed by 1-Nonadecene (13.33%) and 5-Octadecene,(E) (9.37%). The biological activities of these compounds were explained in table 7.

Conventional folk plants are regularly used for their potential healing effects as an alternative of synthetic drugs [44]. Plant bioactive constituents were became an essential approach in solving many health problems by using traditional medicine due to its lesser side-effects and lower costs [45].

TABLE 5. Chemical composition (%) in essential oil analyzed by GC-MS for *Pimpinella anisum in vitro* calli grown on medium elicited by 3.64 mM/l yeast

Compound Name	Retention Time (RT)	Molecular Weight	Molecular Formula	Area%
3-Dodecene,(Z)	13.95	168	$C_{12}H_{24}$	2.48
1-Octanol,2-butyl	14.09	186	$C_{12}H_{26}O$	1.87
Dodecane,2,6,11-trimethyl	16.31	212	$C_{15}H_{32}$	1.86
1-Tetradecene	17.44	196	$C_{14}H_{28}$	5.55
Tetradecane	17.55	198	$C_{14}H_{30}$	1.79
2-Allyl-5-t-butylhydroquinone	19.45	206	$C_{13}H_{18}O_2$	20.32
Nonadecane	19.76	268	$C_{19}H_{40}$	1.96
1-Hexadecanol	20.55	242	$C_{16}H_{34}O$	9.43
Hexadecane	20.64	226	$C_{16}H_{34}$	1.48
1-Decanol,2-hexyl	22.61	242	$C_{16}H_{34}O$	1.57
Heneicosane	22.80	296	$C_{21}H_{44}$	2.91
Cyclobuta[a]dibenzo[c, f]cycloheptadiene,7oxo	23.03	234	$C_{17}H_{14}O$	4.31
5-Octadecene,(E)	23.35	252	$C_{18}H_{36}$	10.44
1-Docosene	25.87	308	$C_{22}H_{44}$	7.88
n-Hexadecyloxy(triethyl)silane	27.73	356	$C_{22}H_{48}OSi$	2.62
Behenic alcohol	28.17	326	$C_{22}H_{46}O$	4.19
1,2-Diphenyl-5-(t-butyl)acephenanthrylene	30.24	410	$C_{32}H_{26}$	9.89
Tetracosyl heptafluorobutyrate	30.54	550	$C_{28}H_{49}F_7O_2$	1.64
Cyclhexane carboxylic acid,1-phenyl,methyl ester	31.35	218	$C_{14}H_{18}O_2$	5.15
Thiocarbamic acid, N,Ndimethyl, S1,3diphenyl2butenyl ester	32.45	311	$C_{19}H_{21}NOS$	2.65

TABLE 6. Chemical composition (%) in essential oil analyzed by GC-MS for *Pimpinella anisum in vitro* calli grown on medium elicited by 5.46 mM/l yeast

Compound Name	Retention Time (RT)	Molecular Weight	Molecular Formula	Area%
Pentadecane (CAS)	15.52	212	$C_{15}H_{32}$	2.38
3-Eicosene,(E)	15.99	280	$C_{20}H_{40}$	2.44
1-Octanol,2-butyl(CAS)	16.14	186	$C_{12}H_{26}O$	2.47
Dodecane,2,6,11-trimethyl	16.32	212	$C_{15}H_{32}$	3.46
Heptadecane	19.08	240	$C_{17}H_{36}$	2.46
2-Allyl-5-t-butylhydroquinone	19.46	206	$C_{13}H_{18}O_2$	6.45
Hexadecane,2,6,11,15-tetramethyl	19.76	282	$C_{20}H_{42}$	3.76
1-Hexadecanol	20.53	242	$C_{16}H_{34}O$	2.30
Heneicosane	22.21	296	$C_{21}H_{44}$	2.89
Octadecane (CAS)	22.80	254	$C_{18}H_{38}$	5.45
2-Hexyl-1-decanol	23.00	242	$C_{16}H_{34}O$	3.37
5-Octadecene,(E)	23.33	252	$C_{18}H_{36}$	9.37
7,9-Di-tert-butyl-1-oxas Piro-(4,5)deca-6,9-diene 2,8-dione	25.12	276	$C_{17}H_{24}O_3$	2.77
Pentacosane (CAS)	25.53	352	$C_{25}H_{52}$	3.53
1-Nonadecene	25.87	266	$C_{19}H_{38}$	13.33
3-Cyano-2-methyl-4-(4methoxyphenyl)-6-[2-bis(methylthio)-ethenyl]pyridine	27.72	342	$C_{18}H_{18}N_2OS_2$	4.10
1-Docosene	28.18	308	$C_{22}H_{44}$	7.12
1,2-Diphenyl-5-(t-butyl)acephenanthrylene	30.25	410	$C_{32}H_{26}$	15.96
Tetracosyl Heptafluorobutyrate	30.55	550	$C_{28}H_{49}F_7O_2$	3.35
5-Methoxy-3,4(2',2',3',3'tetracyanocyclobutano)1,2-dihydro-2,2,4-trimethylquinoline	35.97	331	$C_{19}H_{17}N_5O$	3.04

TABLE 7. Bioactivity of compounds identified in *P. anisum* callus extract

Compound name	Biological effect	Reference
Dodecane,2,6,11-trimethyl	antibacterial activity	Rahbar et al. [30]
Cyclotetradecane	Antimicrobial	Olubunmi and Anthony [31]
2-Allyl-5-t-butylhydroquinone	Antibacterial	*Fung et al. [32]. * Raccach and Henningsen [33]. *Kupp et al. [34]. * Ogunrinola et al. [35].
Hexadecane,2,6,11,15-tetramethyl	Antibacterial activity Antifungal -Antitumor- larvicidal	Sunita et al., [36].
Heneicosane	Microbicide activities	* Vanitha et al. [37]. * Usha et al. [38].
Ecosane	Antifungal- antitumor- antibacterial, larvicidal- antimicrobial- cytotoxic	Sunita et al. [36].
Tricosane	Antibacterial	Sunita et al., [36].
Canthaxanthin	Food-coloring agent- pharmaceutical products	* Zakynthinos and Varzakas, [39]. * Cardoso et al. [40]. * odriguez et al. [41].
17-Pentatriacontene(CAS)	Antioxidant activity	* Yogeswari et al. [42].
Tetratetracontane	Antioxidant activity	Sunita et al. [36].
Phenol,2,4-bis(1,1-dimethylethyl)	Antibacterial activity	*Usha et al. [38].
Nonadecane	Antimicrobial- cytotoxic	Sunita et al. [36].
Octadecane	Lubricants -anticorrosion agents	Sunita et al. [32].
Pentadecane	Sugar phosphatase inhibitor-Antibacterial	Sunita et al. [36]
1,2-Diphenyl-5-(t butyl)acephenanthrylene	Cancer-preventive- flavor analgesic- anti-implantation	Kalpana et al. [43].

The present results of GC-MS analysis, lead to identification of different components present in the acetone extract of anise callus cultures. It was revealed that, except in callus extract grown on medium with 4 mM phenylalanine, 1,2-Diphenyl-5-(t-butyl)acephenanthrylene was mainly found in all treatment as one of the major compounds and reached highest (15.96%) from callus extract grown on medium elicited by 5.46 mM/l yeast. Also, 5-Octadecene,(E) was detected as a main component extracted from P. anisum callus cultures grown on medium elicited by 1.28; 3.46 and 5.46 mM/l yeast with 9.2 %; 9.89% and 9.37% respectively. Furthermore, newly identified compound were found in callus extract developed on medium with phenylalanine as 2-Allyl-5-t-butylhydroquinone (17.38%); 1-Hexadecanol (8.84%);1-Docosene (8.31%); Cyclohexane carboxylic acid, 1-phenyl, methyl ester (6.01%); Ethane, 1,2diethoxy (24.56%) followed by Butylated Hydroxytoluene (21.82%) and Carvone (14.88%) and 1-Octadecanol(CAS) was presented in highest amount (15.66%). While the callus extract grown on medium elicited by yeast 1-Docosene (17.40%);1-Hexadecanol (9.43%) and 1-Nonadecene (13.33%) were also detected . So far no published reports regarding the effect of yeast and phenylalanine on bioactive constituents of anise calli cultures by GC-MS analysis. As shown in table (7), many different compounds are having some important therapeutic principles for progressing drug sighting.

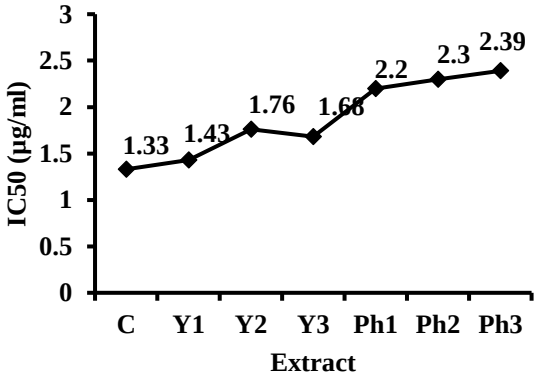

Fig.1. Cytotoxic activity (IC50) of *Pimpinella anisum* calli extracts grown on different yeast and phenylalanine concentrations on MCF7 cell line. Tested yeast concentrations were Y1: 1.82; Y2: 3.64 and Y3: 5.46 mM/l. phenylalanine concentrations were Ph1: 2; Ph2:4 and Ph3: 6 mM/l. free yeast and phenylalanine medium (C) was used as control medium.

Antibreast cancer activity:

This work was carried out by SRB method using the inhibition percent of carcinoma cells for breast and their IC50 values. Effectiveness of the different extracts derived from anise shoot tip calli against tumor activity has reported. In this survey, authors provide a brief and overall update for the functional role of fatty acids and their derivatives in reduction of the breast cancer cell viability (Figure 1). The data demonstrated that at 3.64 mM/l of yeast concentration, the callus extract inhibited the vitality

of MCF7-breast cancer cells to be IC50 of 1.76 µg/ml, whereas at 6 mM/l of phenylalanine concentration, the calli extract showed the highest effect on cancer cell growth with IC50 value of 2.39 µg/ml; compared with control treatment of 1.33 µg/ml (Figure 1).

Based on our results, it could be concluded the vital role of volatile oils to raise the efficiency of anise shoot tip callus extracts to inhibit the breast cancer cell growth. Furthermore, it should be paid attention for the presence of many active constituents as Hexadecane,2,6,11,15-tetramethyl ; Ecosane ; Canthaxanthin ; Nonadecane and 1,2-Diphenyl-5-(t-butyl)acephenanthrylene regarding their antitumor activity (Table 7). Our findings are comparatively in similarity with data published before [46-49].

Conclusions

The results of the present research confirmed a significant biological activity of *Pimpinella anisum* calli extracts. Detection of different active constituents has potential to find out new sources for functional drugs. This study will provide base-line data for further detailed investigations of various biological activities of this plant and of its use as a functional food.

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References

[1] Prabhadevi V., Sahaya S.S., Johnson M., Venkatramani B. and Janakiraman N., Phytochemical studies on Allamanda cathartica L. using GC-MS. *Asian Pacific J. Trop. Biomed.*, 2(2), 550–554(2012).

[2] Hasan P., Yasa N., Ghanbari S.V., Mohammadirad A., Dehghan G. and Abdollahi M., *In vitro* antioxidant potential of Teucrium polium as compared to α- tocopherol. *Acta Pharm.*, 57, 123-129 (2007).

[3] Somnath D., Akalanka D., Sudhakar babu, A.M.S., Siddabathuni A., *Pharmacognosy Journal*, 5,265-268(2013).

[4] Nostro A., Germano M.P., Dangelo V., Marino A., cannatelli M.A. *Letapplmicrobial*, 30,379-384(2000).

[5] Arokiyaraj S., Radha R., Martin S., Perinbam K., *Indian j Sci Technol*, 1,1-4(2008).

[6] Yang W., Lu J., Weng J., et al. China National Diabetes and Metabolic Disorders Study Group. Prevalence of diabetes among men and women in China. *N Engl J Med.* 362. 1090-1101(2010).

[7] Özcan M.M. and Chalchat C.J. Chemical composition and antifungal effect of anise (*Pimpinella anisum* L.) fruit oil at ripening stage. *Ann. Microbiol.*, 56, 353-358(2006).

[8] Tirapelli C.R., Andrade R.C., de Cassano O.A., de Souza A.F., Ambrosio R.S., Costa B.F. and da Oliveria, M.A. Antispasmodic and relaxant effects of the hydroalcoholic extract of *Pimpinella anisum* (Apiaceae) on rat anococcygeous smooth muscle. *J. Enthopharmacol.*, 110, 23-29(2007).

[9] Omidbaigi R., Hadjiakhoondi A., Saharkhiz M. Changes in content and chemical composition of *Pimpinella anisum* oil at various harvest time. *J. Essent. Oil Bearing Plants*, 6 (1), 46-50(2003).

[10] Embong M.B., Hadzie D., Molnar S. Essential oil in Alberta anise oil (*Pimpinella anisum* L.). *Can. J. Plant Sci.*, 59, 681-688(1977).

[11] Ashraf M., Siddiqui M.A., Bhatty M.K. Studies on the essential oils of the Pakistan species of the family Umbelliferae. *Pakistan J. Sci. Ind. Res.*, 23, 211-212(1980).

[12] Ivanic R., Savin K., Robinson F.V. Essential oil from *Pimpinella serbica* fruits. *Planta Med.*, 48 (1), 60-61(1983).

[13] Lawrence D.M. Progres in essential oils [Anise oil, Calamus oil, Cardamom oils]. *Perfumer and Flavorist*, 8, 63-65(1984).

[14] Baser K.H.C., Özek T. Essential oil of *Pimpinella aromatic* Bieb. from Turkey. *J. Essent. Oil Res.*, 8, 463-464(1996).

[15] Rodrigues V.M., Rosa P.T.V., Marques M.O.M., Petenale A.S., Meireles M.A.A. Supercriticial extraction of essential oil from aniseed (*Pimpinella anisum* L.) using CO2 solubility, kineticsand composition data. *J. Agric. Food Chem.*, 51, 1518-1523(2003).

[16] Askari F., Sefidkon F., Mirza M. Quantitative and qualitative study of components in essential oil of *Pimpinella anisum* L. *Journal of Pajouhes –Va- Sazandegi*, 38, 70-77(1998).

[17] Askari F., Sefidkon F., Mozafarian V. Essential oil composition of *Pimpinella aurea* D.C. from Iran. *Flavour Fragr. J.*, 20,115-117(2005).

[18] Chong, T.M., Abdullah, M.A., Lai, Q.M., Nor'Aini, F.M., Lajis, N.H. Effective elicitation factors in *Morinda elliptica* cell suspension culture. *Process Biochem*., 40,3397–3405(2005).

[19] Smetanska, I. Production of secondary metabolites using plant cell cultures. *Adv. Biochem. Eng. Biotechnol.*, 111,187–228(2008).

[20] Arora R. Medicinal Plant Biotechnology. CABI, Wallingford, Oxford-shire, UK (2010).

[21] Díaz-Valle A., López-Calleja A.C. and Alvarez-Venegas R. Enhancement of Pathogen

Resistance in Common Bean Plants by Inoculation with *Rhizobium etli*. *Front. Plant Sci.* 10,1317 (2019). doi: 10.3389/fpls.2019.01317

[22] Marzieh R.S., Abdollah G. P., Fariborz M., Ahmad R. G. L-Phenylalanine and bio-fertilizers interaction effects on growth, yield and chemical compositions and content of essential oil from the sage (*Salvia officinalis L.)* leaves. *Industrial crops and Products*, 137, 1-8 (2019).

[23] Mansi S., Kamaljit G., Rupali J., Daizy R. B.,Harminder P.S. and Ravinder K. K. Essential oils as anticancer agents: Potential role in malignancies, drug delivery mechanisms, and immune system enhancement. *Biomedicine & Pharmacotherapy*. 146, 112514 (2022).

[24] Nermeen M. A. and Usama I.A., Induced biosynthesis of acephenanthrylene in callus culture of *Pimpinella anisum* L., by yeast and phenylalanine application. *Egypt. J. Chem.*, (in press).

[25] Murashige, T., Skoog, F.A. A revised medium for a rapid growth and bioassays with tobacco tissues cultures. *Plant Physiol.*, 15,473-479 (1962).

[26] Diwan R., Malpathak N. Bioprocess optimization of furanocoumarin elicitation by medium renewal and re-elicitation: a perfusion-based approach. *Appl. Biochem. Biotechnol.*, 163(6),756–764(2011).

[27] Mc Lafferly F.W., Registry of mass spectral data, Ed. Wiley New York. (1989).

[28] Stein S.E., National Institute of Standards and Technology (NIST) Mass Spectral Database and Software, Version 3.02, USA. (1990).

[29] Suresh P.K., Vijaybabu M.R., Arunkumar A., Arunakaranl J. Anticancer effects of ethanolic neem leaf extract on prostate cancer cell line (PC-3). *J. Ethnopharmacol*., 105,246–250(2006).

[30] Rahbar N., Shafagha A., Salimi F. Antimicrobial activity and constituents of the hexane extracts from leaf and stem of Origanum vulgare L. sp. Viride (Boiss.) Hayek. Growing wild in Northwest Iran. *J. Med. Plants Res.*, 6, 2681–2685(2012).

[31] Olubunmi A. W. and Anthony J.A. Chemical Constituents and Biological Activities of Essential Oils of *Hydnora africana* Thumb Used to Treat Associated Infections and Diseases in South Africa. *Appl. Sci.*, 7(5), 443(2017).

[32] Fung D.Y.C., Lin C.C.S., Gailani M.B. Effect of phenolic antioxidants on microbial growth. *Crit. Rev. Microbiol.*, 12,153–83(1985).

[33] Raccach M. and Henningsen E.C. Antibacterial effect of tertiary butylhydroquinone against 2 genera of Gram-positive cocci. *J. Food Sci.*, 47, 106–9(1982).

[34] Kupp L.I., Rosen S., Beck F.M. Effect of anti-oxidants on growth and lactic acid production by Streptococcus mutans. *J. Den.t Res.*, 64, 1016–8(1985).

[35] Ogunrinola O.A., Fung D.Y.C., Jeon I.J. Escherichia coli O157:H7 growth inlaboratory media as affected by phenolic antioxidants. *J. Food Sci.*, 61, 1017–21(1996).

[36] Sunita A., Ganesh K., Sonam M. Gas chromatography-mass spectroscopy analysis of root of an economically important plant, *Cenchrus ciliaris* l. from thar desert, rajasthan (INDIA). *Asian Journal of Pharmaceutical and Clinical Research*, 10(9), -64-69(٢٠١٧). doi:10.22159/ajpcr.2017.v10i9.19259.

[37] Vanitha V., Vijayakumar S., Nilavukkarasi M., Punitha V. N., Vidhya E. and Praseetha P. K. Heneicosane, A novel microbicidal bioactive alkane identified from Plugmbago zeylanica L. *Industrial Crops and Products*, 154, 112748(2020).

[38] Usha N. S., Sangareshwari S., Lata K. Gas chromatography-mass spectrometry analysis of bioactive constituents from the marine Streptomyces. *Asian J. Pharm. Clin. Res.*, 8,244–246(2015).

[39] Zakynthinos G. and Varzakas T. Carotenoids: From plants to food industry. *Curr. Res. Nutr. Food Sci.*, 4,38–51(2016).

[40] Cardoso L.A.C., Karp S.G., Vendruscolo F., Kanno K.Y.F., Zoz L.I.C., Carvalho J.C. Biotechnological production of carotenoids and their applications in food and pharmaceutical products. In Carotenoids; Cvetkovic, D., Nikolic, G., Eds.; In tech. Open, London, UK, 8, 125–141(2017).

[41] Odriguez-Concepcion M., Avalos J., Bonet M.L., Boronat A., Gomez-Gomez, L., Hornero-Mendez D., Limon M.C., Meléndez-Martínez A.J., Olmedilla-Alonso B., Palou A. et al. A global perspective on carotenoids: Metabolism, biotechnology, and benefits for nutrition and health. *Prog. Lipid Res.*, 70, 62–93(2018).

[42] Yogeswari S., Ramalakshmi S., Neelavathy R., Muthumary J. Identification and comparative studies of different volatile fractions from Monochaetia kansensis by GCMS. *Glob. J. Pharma*., l6,65–71(2012).

[43] Kalpana D. R., Vasantha S., Panneerselvam A., Rajesh N.V., Jeyathilakan N., Perumal P., Rajasekar D. Gastrothylax crumenifer: Ultra structure and histopathology study of *in vitro* trematodicidal action of Marattia fraxinea (Sm.), *Clinical Phytoscience*, 3,3(2017).

[44] Rivera J.O., Loya A.M., Ceballos, R. Use of Herbal Medicines and Implications for Conventional Drug Therapy Medical Sciences.

Alternative and Integrative Medicine, 3,2-6(2014).

[45] Zhu W., Zhang Y., Huang Y., Lu L. Chapter twelve – Chinese herbal medicine for the treatment of drug addiction. *Int. Rev. Neurobiol.,* 135, 279-295(2017).

[46] Dichter M.A., Delanty N. Antioxidant therapy in neurological disease. *Arch. Neurol.,* 57,1265–126(2000).

[47] Kabelitz N., Santos, P.M., Heipieper, H.J. Effect of aliphatic alcohols on growth and degree of saturation of membrane lipids in Acinetobacter calcoaceticus. *FEMS Microbiol. Lett.*, 220,223–227(2003).

[48] Odenigbo U.M. and Otisi C.A.O. Fatty acids and phytochemical contents of different coconut seed flesh in Nigeria. *Int. J. Plant Physiol. Biochem.,* 3,176–182(2011).

[49] Silva M.J.D., Carvalho A.J.S., Rocha C.Q., Vilegas W., Silva M.A., Gouvea C.M.C.P. Ethanolic extract of Mimosa caesalpiniifolia leaves: chemical characterization and cytotoxic effect on human breast cancer MCF-7 cell line. *S. Afr. J. Bot.,* 93, 64–69(2014).