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Gas Chromatography/Mass Spectrography (GC/MS) Analysis and Biological Properties of Probiotic Fermented *Solenostemma argel*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Solenostemma argel (*S. argel*) is an important medicinal plant in Egypt and Sudan, with antitumor effects. This study objective to elucidate the chemometric profile and antitumor properties of *S. argel* extract and the effect of fermentation on its properties. *S. argel* was fermented by two probiotic strains *Lactobacillus rhamnosus* and *Lactobacillus acidophilus*. While fermentation reduced total phenolics, and total flavonoid contents as well as DPPH radical scavenging activity, it enhanced antitumor activity on Ehrlich ascites carcinoma cell. The GC/MS analysis of fermented and non-fermented *S. argel* revealed the formation of new compounds of known antitumor activity.

Keywords: Solenostemma argel; probiotic fermentation; GC/MS; antitumor activity.

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1. INTRODUCTION

The plant *Solenostemma argel* (*S. argel)* belongs to the *Asclepiadaceae* family mainly located in the tropics to subtropic regions, especially in Africa and South America. It is a desert plant widely distributed in Egypt (Wadi Allaqui) with common name 'Hargal'. The plant is widely used in traditional folkloric medicine as antispasmodic [1], anti-inflammatory and anti-rheumatic agent [2]. Smoke inhalation and infusion of the whole plant is used in treatment of diabetes mellitus, hypercholesterolemia, jaundice, cough, cold and measles. It was described to alleviate gastrointestinal cramp, urinary tract infection and menstrual disturbance, in addition it was shown to possess anti-syphilitic properties when used for a long period of time [3].

This family is a rich source of indoline, alkaloids, steroids, steroidal alkaloids, pregnanes and their glycosides which possess antitumor and anticancer activities [4,5]. Previous phytochemical studies showed that the leaves are rich in carbohydrates, potassium, calcium, magnesium, and sodium while low in fibers, protein, oil, ash, copper, ferrous, manganese, and lead [6]. Moreover, investigations revealed the presence of chemical ingredients such as pyrgene glycosides, flavonoids, kaempferol, quercetin, rutin, flavonols, flavanones, chalcones and alkaloids [7,8,9]. From all these ingredients, pyrgene glycosides were reported to reduce cell proliferation [9].

It is well documented that, fermentation process is used to produce some important products in an industrial scale by decomposing organic materials in absence of oxygen [10]. Microbial strains used in fermentation process are lactic acid bacteria, such as *Bifidobacterium* sp*.* and *Lactobacillus* sp*.,* and some yeast, such as *Saccharomyces* sp*.* [10]. Microbial strains used in fermentation are able to convert some of food or plant components like sugars to alcohols and lactic acid, that may increase components bioactivity [10].

It was hypothesized that the bioactivity of *S. argel* could be increased by fermentation with Lactic acid bacteria. Therefore, this study objective was to evaluate the effect of fermented *S. argel* on Ehrlich acite tumor cells in vitro and to identify transformed chemical constituents.

2. MATERIALS AND METHODS

2.1 Microorganisms and Culture Conditions

The lactic acid bacteria (LAB) strains used in this study were: *Lactobacillus rhamnosus,* ATCC 7469 and *Lactobacillus acidophilus*, ATCC 4356. All cultures were stored at −25ºC in liquid MRS (de Man, Rogosa and Sharpe Lactobacilli media) (Oxoid, Basingstoke, Hampshire, UK) with 20% sterile glycerol.

2.2 Probiotic Fermentation of *Solenostemma argel* Leaves

Solenostemma argel leaves were purchased from the local market and allowed to shade dried at room temperature and grinded. The *S. argel* leaves powder was fermented by 2% mixed culture of *L. rhamnosus* and *L. acidophilus* for 24 hrs at 37ºC.

2.3 Plant Extracts Preparation

Ten solvents (methanol, ethanol, diethyl ether, acetone, ethyl acetate, dichloromethane, cold water, chloroform, hexane and hot water) were used to prepare 20 plant extracts. 30 gram of plant materials were extracted by immersing them in 150 ml of the solvent for 48 hrs at room temperature under dark conditions, then filtered through clean muslin cloth followed by a filter paper, the process was repeated again for another 24 hrs. The extracts were concentrated under vacuum by rotary evaporator at 40ºC. The dry extracts were stored at -80ºC.

2.4 Viability of Ehrlich Ascites Carcinoma Cells (EACC) Using MTT Assay Exclusion Technique

The effect of fermented and non-fermented plant extract on Ehrlich ascites carcinoma cells (EACC) was determined by tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetra-zolium bromide proliferation assay [11]. A cell line of Ehrlich ascites carcinoma was obtained from The National Cancer Institute (NCI), Cairo, Egypt, has been used. The cell line was maintained in female Swiss albino mice by weekly intraperitoneal (ip) transplantation of 2.5×10^6 cells. The EACC cells were aspired from transplanted animals after ≈ 7 days of transplantation. The EACC cells were centrifuged at 1000 rpm for 5 min and washed with saline.

Briefly, 0.2 ml of (2×10^7 cells/ml) EACC suspension was seeded in each well of 24-well plates. 0.2 ml of fermented (1 mg/ml in 0.1% dimethyl sulfoxide) or non-fermented extracts (1 mg/ml in water) was added and cells were incubated for 24 hrs at 37ºC, 5% CO_2 with 98% relative humidity. The medium was replaced with medium containing 0.5 mg/ml of 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) for 2 hrs. The supernatant was aspirated and MTT-formazan crystals were dissolved in 0.5 ml of a mixture of iso-propanol and 0.1 N HCL and change in color was measured spectrophotometrically at 560 nm. Percent growth inhibition of cells exposed to treatments was calculated as follows:

% Inhibition = 100 − (Abs. of Sample / Abs. of Control × 100)

2.5 Determination of Phytoconstituents

2.5.1 Preparation of sample

100 mg of the methanolic extract powder of each sample was re-dissolved in 5.0 ml of methanol.

2.5.2 Determination of total phenolics content

The total phenolics content (TPC) of the crude extracts was determined using the method of [12] with slight modifications. To 0.5 ml of plant extract, 1.5 ml of Folin-Ciocalteau reagent was added (1:10 v/v diluted with distilled water), allowed to stand for 5 min. After 5 min, 2.0 ml of sodium carbonate 7.5% were added. These mixtures were incubated for 90 min in the dark with intermittent shaking. After incubation development of blue color was observed. Finally absorbance of blue color in different samples was measured at 725 nm. The phenolics content was expressed as mg/g caffeic acid equivalent.

2.5.3 Determination of total flavonoid content

The total flavonoid content (TFC) of the crude extracts was determined using the colorimetric aluminum chloride assay. An aliquot (0.5 ml) of extracts were added to 2ml of distilled water followed by the addition of 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v) and allowed to stand for 6 min. Later 0.15 ml of aluminum trichloride (10% $AlCl_3$) was added and incubated for 6 min, followed by the addition of 2 ml of sodium hydroxide (NaOH, 4% w/v) and volume was made up to the 5 ml with distilled water. After 15 min of incubation the mixture turns to pink whose absorbance was measured at 510 nm. The TFC was expressed in mg of luteolin equivalents per gram of extract [13].

2.6 DPPH Radical Scavenging Activity

The scavenging activity to free-radicals was measured by using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay. DPPH assay was performed according to [14] Gyamfi et al. DPPH solution was prepared by dissolving 3.2 mg of DPPH in 100 ml of 82% methanol. 2.8 ml of DPPH solution were added to glass vial with 0.2 ml of plant methanol extract of different concentrations, to give final concentration of 31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml. The mixture was mixed well and kept in the dark at room temperature (25–28ºC) for 1 h. Change in mixture color was measured at 517 nm. Mixture of 2.8 ml of 82% methanol and 0.2 ml of methanol were used as blank while 0.2 ml of methanol and 2.8 ml of DPPH solution were taken as control. The test of each fraction was performed in triplicate. Percentage inhibition was calculated according to the following formula:

% Scavenging = Abs. of Control – Abs. of Sample / Abs. of Control × 100.

IC_{50} value was calculated by graph pad prism software.

2.7 Gas Chromatography/ Mass Spectrography (GC/MS)

GC/MS analysis was carried out using (Agilent Technologies 7890 A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5 ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 µm film thickness). The carrier gas was helium with the linear velocity of 1ml/min.

Interpretation of mass spectrum of GC/MS was accomplished using NIST and WILEY library Mass Spectral Database (NIST 11), with NIST MS search program v.2.0 g (National Institute Standard and Technology (NIST). The identification of components was based on a comparison of the mass spectra of unknown component and retention time with those of known components stored in the NIST library. The molecular weight, name and structure were ascertained.

2.8 Statistical Analysis

Experimental data were analyzed by one way analysis of variance (ANOVA) using SPSS (statistical package for social sciences, version 15, Inc, Chicago, IL, USA) and results were represented as mean ± SE.

3. RESULTS

3.1 Ehrlich Ascites Carcinoma cells (EACC) % Inhibition Using MTT Assay

Non-fermented and fermented *S. argel* leaves were extracted using 10 different solvents (methanol, ethanol, diethyl ether, acetone, ethyl acetate, dichloromethane, cold water, chloroform, hexane and hot water). Each extract was evaporated and concentrated. Ehrlich ascites carcinoma antitumor activity for each extract was investigated by MTT assay. Ehrlich ascites carcinoma cells (EACC) incubated with different extracts of *S. argel* showed different cytotoxic activity against EACC according to the solvent. Methanol, cold water and ethyl acetate extracts induced growth inhibitory effect by 15.15%, 12.69% and 14.01% respectively compared to the control. Fermented *S. argel* (F*S. argel*) extracts of acetone, ethyl acetate, and methanol inhibited cells growth by 36.02%, 35.07%, 33.29% respectively compared to control. Accordingly it is observed that methanol extracts of *S. argel* exhibits high antitumor activity which markedly increased almost two folds by fermentation of *S. argel*.

3.2 GC/MS Chemometric Profile

GC-MS analysis of the methanol extract of *S.argel* revealed 25 different compounds (Table 1, Fig. 3) by comparison of their mass fragmentation pattern by NIST database library**.** The compounds are: Xylitol (35.65%), 4-Methylcatechol (23.89%), Sinapic acid (8.13%), Linalool acetate (4.28%), α-Copaene (3.21%), 2-(But-2'-enyl) phenol (2.85%), Menthol,1'-(butyn-3-one-1-yl)-, (1S,2S,5R)-(2.85%), α-Elemene (2.14%), and Junipene (2.14%), were found to be the major constituents, while, 7-methoxy-3-(4-methoxyphenyl)-2,3-dihydro-4H-chromen-4-one (2.14%), β-Guaiene (1.6%); β-Asarone (1.6%), Phenol, 4-(3-hydroxy-1-propeenyl)-2-methoxy-(1.5%), Isoferulic acid (1.43%), γ-Himachalene (1.25%), L-Aspartic acid (1.07%), Resorcinol (0.71%), Valproic acid (0.71%), 6-monohydroxyflavone (0.53%), Tricosanoic acid (0.53%), Apigenin-7-glucoside (0.53%), 3,7,3',4'-tetra-O-methyl-5-O-(trimethylsilyl) quercetin (0.36%), Phenol, 2-methoxy-5-(1-propenyl)-(0.36%), α-Terpineol (0.36%), and 6-Hydroxyflavone (0.18%), were found to be present in minor amount.

On the other hand, GC-MS analysis of the methanol extract of F S. argel revealed 36 different compounds (Table 1, Fig.3). Among these Terpinyl formate (13.0%), Cumol (12.21%), L-Aspartic acid (12.03%), Xylose (10.82%); Acetic acid (6.11%), Pentanoic acid (5.55%), 2-hexadecanol (4.44%), Hexadecanoic acid, methyl ester (4.26%), Resorcinol (3.33%), Kynurenic acid (3.33%), Phenol, 2, 3, 5, 6 tetramethyl- (3%), Bornate (2.04%) were found to be present in major amounts, whereas, Butanoic acid, 3-hydroxy-(0.46%), 4- Albuterol (0.37%), 2-Decenoic acid (1.58%), 5-Aminovaleric acid (1.48%), Salbutamol (1.48%), Geranyl-p-cymene (1.48%) , Limonene-1, 2-diol (1.33%), Coumarin,7,8-dihydro-7-hydroxy-6-methoxy-8-oxo-(1.3%), α-Tocopherol (1.3%),

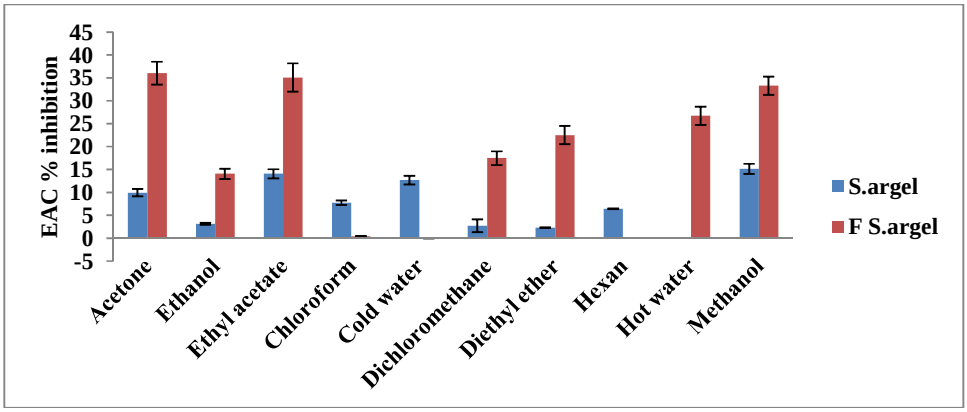


Fig. 1. Inhibition % of Ehrlich ascites carcinoma cells (EACC)

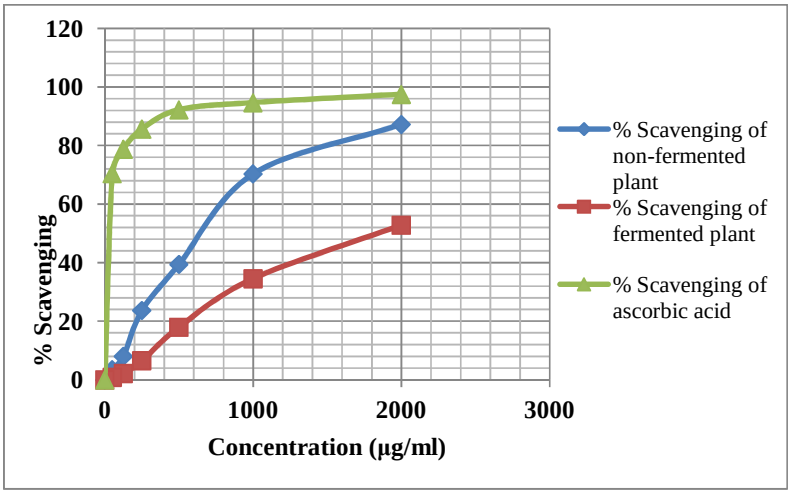


Fig. 2. The antioxidant activity of *S. argel* and fermented *S. argel* methanol extracts.

3-Hydroxyflavone (0.18%), 1,8-Cineole (1.30%), Hydroquinone (1.11%), Methylcatechol (1.11%), Scopoletin (0.93%), β-Curcumene (0.74%), γ-Himachalene (0.74%), Valproic acid (0.56%), Esculetin (0.56%), 3,4-Dimethoxycinnamic acid (0.56%), Apgenin 7-glucoside (0.53%), Antioxidant No. 33(0.37%), β-Guaiene (0.37%), Geranyl isovalerate (0.19%), Cis-13-Eicosenoic acid (0.14%), and p-Coumaric alcohol (0.09%) were found to be present in trace amounts.

3.3 Total Phenolic and Flavonoids Content

The total phenolics content of *S. argel* 0.804 expressed as mass of caffeic acid equivalent per mass of extract (mg caffeic/g extract) was not significantly affected by fermentation and was 0.784 in F *S. argel*. Meanwhile, the luteolin content of *S. argel* 2.023 mg/g extract was significantly decreased by fermentation to 1.195 mg /g extract F *S. argel*.

3.4 DPPH Radical Scavenging Activity

The antioxidant activity and flavonoids content of *S. argel* and F*S. argel* extracts measured by DPPH radical scavenging activity describe extracts ability to scavenge reactive oxygen species and lipid peroxyl. Fermentation process markedly decreased the antioxidant activity of methanol extracts from 70.37% in *S. argel* to 34.57 % in F*S. argel* (Fig. 2).

3.5 Inhibitory Concentration (IC_{50})

The inhibitory concentration IC_{50} was markedly increased two folds by fermentation to be 1.85 mg/ml of F*S. argel* compared to 0.65 mg/ml for *S. argel*.

Identification of methanol extracts chemical compounds content of *S. argel* and F*S. argel* using GC/MS showed marked changes due to fermentation process in the identified chemical compounds of F*S. argel* compared to native chemical compounds of *S. argel*, Fig. (a,b), (Table 1).

GC-MS analysis showed that, 4 compounds were not affected by fermentation and they are: 4-methylcatechol, L-aspartic acid, Resorcinol, and γ-Himachalene. Fermentation process induced the formation of 36 new compounds and derivatives from the original 25 compounds in the original plant methanol extract.

4. DISCUSSION

Fermentation; Biotechnological process, can maintain and improve the nutritional, safety, sensory, and shelf-life properties of many food products including plant food products [15]. Most plant products can be lactic acid fermented leading to alterations in secondary metabolites composition of the plant, that affect product properties such as antioxidant activity [16].

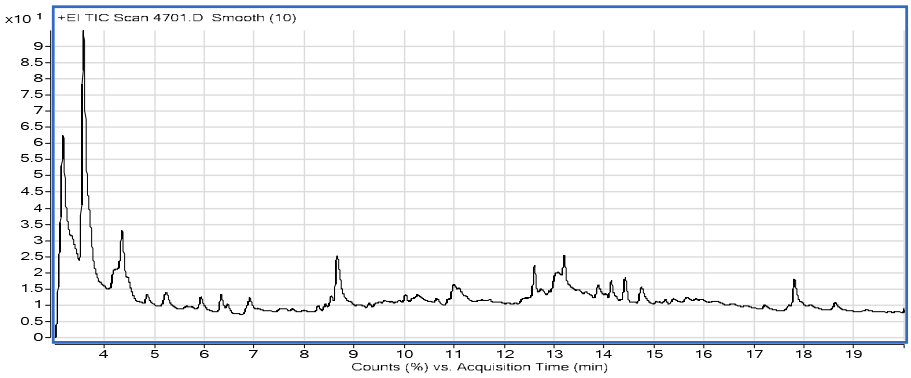
Microbial strains selection was based on the ability of microbial strains to be active and attain in a nutrient limitations matrix, presumably sugars or free amino nitrogen, in a non pH controlled fermentation. Lactobacilli are very fastidious micro-organisms that generally require fermentable carbohydrates, amino acids, vitamins of the B-complex, nucleic acids and

minerals to grow [17]. Thus, the substrate composition and nutritional requirements of the strain considerably affect the overall performance of the fermentation.

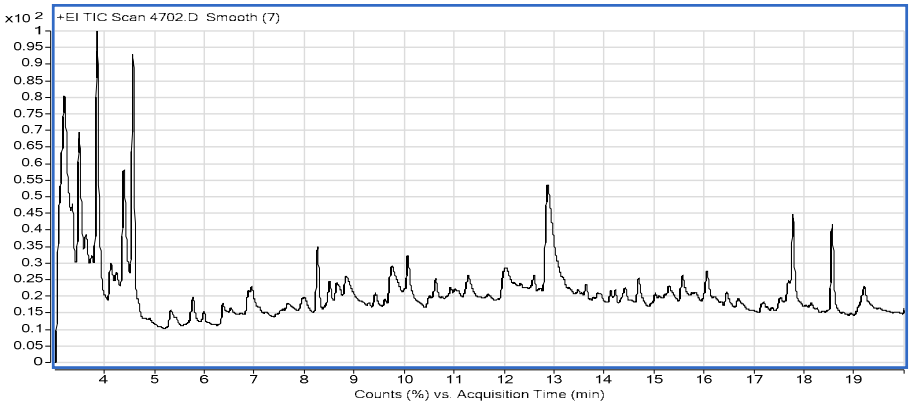
There is no single solvent which could extract all the antioxidants from plants due to their variation in solubility and polarity. In the present study, methanol was used as a solvent as it has high polarity. The results showed a slight increase in the extraction yield of the fermented *S. argel* methanol extract than that of the non fermented *S. argel* methanol extract from 10.8% to 12.3% after 48 hrs of fermentation period. Similar results also were found with wheat (*Triticum aestivum* Linn.) fermented with fungi (*Cordyceps militaris*) [18]. Fermentation induced structural breakdown of plant cell walls, leading to the enhancement of the extraction yield.

Many studies indicated that fermentation causes enhancement of total phenolics and flavonoids content [17], meanwhile, the present study showed that there was a decrease in the total phenolics and flavonoids content in F *S. arge*l methanol extract compared to non fermented one. The degradation of phenolic compounds during fermentation occurred by enzymatic process including polyphenol-oxidase (PPO) and through a non-enzymatic process [19].

Usually, antioxidant activity is directly proportional to the total phenolics and flavonoids content in plant. DPPH is a relatively stable free radical that is neutralized by antioxidants of plants due to their hydrogen radical donating ability [20](Sreejayan and Rao, 1996). In the present study, probiotic fermentation decreased the percentage of DPPH radical scavenging activity compared to that of ascorbic acid. This decrease in antioxidant activity could be linked to the decrease in total phenolics and flavonoids similar conclusion was attained by Hunaefi et al. [21].



(a) Non-fermented *S. argel*



(b) Fermented *S. argel*

Fig. 3. Typical GC-MS chromatogram of (a) non-fermented and (b) fermented *S. argel*

Table 1. Chemical composition of fermented and non-fermented methanol extract of *S. argel* using GC-MS analysis

No.	Compound	R.T.	Molecular formula	Molecular weight	FSA	NSA	% Peak
1-	Xylitol	3.19	$C_5H_{12}O_5$	152.15		+	35.65
2-	Xylose	3.21	$C_5H_{10}O_5$	150.13	+		10.82
3-	Butanoic acid, 3-hydroxy-	3.36	$C_4H_8O_3$	104.10452	+		0.46
4-	Acetic acid	3.50	$C_2H_4O_2$	60.05	+		6.11
5-	4-methylcatechol	3.58, 3.63	$C_7H_8O_2$	124.13	+ (3.63)	+ (3.58)	FSA (1.11) NSA (23.89)
6-	Albuterol	3.74	$C_{13}H_{21}NO_3$	239.32	+		0.37
7-	Cumol	3.85	C_9H_{12}	120.19158	+		12.21
8-	5-Aminovaleric acid	4.13	$C_5H_{11}NO_2$	117.14634	+		1.48
9-	Sinapic acid	4.36	$C_{11}H_{12}O_5$	224.21		+	8.13
10-	Valproic acid	4.38	$C_8H_{16}O_2$	144.21144	+		0.56
11-	Pentanoic acid	4.57	$C_5H_{10}O_2$	102.1317	+		5.55
12--	L-aspartic acid	4.66, 4.88	$C_4H_7NO_4$	133.10	+ (4.66)	+ (4.88)	FSA (12.03) NSA (1.07)
13-	2-Decenoic acid	5.34	$C_{10}H_{18}O_2$	170.248703	+		1.85
14-	Isoferulic acid	5.93	$C_{10}H_{10}O_4$	194.184		+	1.43
15-	Esculetin	6.00	$C_9H_6O_4$	178.14	+		0.56
16-	β- Asarone	6.33	$C_{12}H_{16}O_3$	208.2536		+	1.60
17-	Scopoletin	6.36	$C_{10}H_8O_4$	192.16812	+		0.93
18-	6-hydroxyflavone	6.47	$C_{15}H_{10}O_3$	238.24		+	0.18
19-	3-hydroxyflavone	6.52	$C_{15}H_{10}O_3$	238.2381	+		0.19
20-	7-methoxy-3-(4-methoxyphenyl)-4H-chromen-4-one	6.90	$C_{17}H_{16}O_4$	284.312		+	2.14
21-	Salbutamol	7.99	$C_{13}H_{21}NO_3$	239.32	+		1.48
22-	Resorcinol	8.25, 8.29	$C_6H_6O_2$	110.1	+ (8.25)	+ (8.29)	FSA (3.33) NSA (0.71)
23-	6-monohydroxyflavone	8.43	$C_{15}H_{10}O_3$	238.2381		+	0.53
24-	Hydroquinone	8.52	$C_6H_6O_2$	110.11	+		1.11
25-	Linalool acetate	8.65	$C_{12}H_{20}O_2$	196.286		+	4.28
26-	Tricosanoic acid	9.30	$C_{23}H_{46}O_2$	354.61014		+	0.53
27-	3,4-dimethoxycinnamic acid	9.42	$C_{11}H_{12}O_4$	208.2106	+		0.56
28-	Kynurenic acid	9.77	$C_{10}H_7NO_3$	189.168	+		3.33
29-	Valproic acid	10.02	$C_8H_{16}O_2$	144.21144		+	0.71
30-	Bornate	10.09	$C_{13}H_{19}NO_2S$	253.36046	+		2.04
31-	1,8-cineole	10.63	$C_{10}H_{18}O$	154.249	+		1.30
32-	Apigenin 7-glucoside	10.66	$C_{21}H_{20}O_{10}$	432.38		+	0.53
33-	3,7,3',4'-tetra-O-methyl-5-O-(trimethylsilyl) quercetin	10.89	$C_{22}H_{26}O_7Si$	430.523		+	0.36
34-	Geranyl isovalerate	10.92	$C_{15}H_{26}O_2$	238.37	+		0.19
35-	2-(But-2'-enyl) phenol	10.99	$C_{10}H_{12}O$	148.20468		+	2.85
36-	Limonene-1,2-diol	11.29	$C_{10}H_{18}O_2$	170.24872	+		1.33
37-	Phenol, 2, 3, 5, 6 tetramethyl-	12.03	$C_{10}H_{14}O$	150.2176	+		3.00
38-	ρ-Coumaric alcohol	12.33	$C_9H_{10}O_2$	150.1745	+		0.09
39-	β-Curcumene	12.59	$C_{15}H_{24}$	204.35628	+		0.74
40-	α –Copaene	12.61	$C_{15}H_{24}$	204.3511		+	3.21
41-	Terpinyl formate	12.88	$C_{11}H_{18}O_2$	182.27	+		13.00
42-	Phenol,2-methoxy-5-(1-propenyl)-	12.89	$C_{10}H_{12}O_2$	164.083725		+	0.36
43-	α-Terpineol	13.06	$C_{10}H_{18}O$	154.24932		+	0.36
44-	Junipene	13.20	$C_{15}H_{24}$	204.355		+	2.14
45-	γ-Himachalene	13.87, 13.89	$C_{15}H_{24}$	204.35106	+ (13.87)	+ (13.89)	FSA (0.74) NSA (1.25)
46-	β-Guaiene	14.14	$C_{15}H_{24}$	204.3511	+	+	FSA (0.37)

No.	Compound	R.T.	Molecular formula	Molecular weight	FSA	NSA	% Peak
							NSA (1.60)
47-	Antioxidant No.33	14.22	$C_{14}H_{22}O$	206.32388	+		0.37
48-	α – Elemene	14.41	$C_{15}H_{24}$	204.35		+	2.14
49-	(+)-α-Tocopherol	14.68	$C_{29}H_{50}O_2$	430.71	+		1.30
50-	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-	14.74	$C_{10}H_{12}O_3$	180.2005		+	1.50
51-	Cis-13-Eicosenoic acid	15.02	$C_{20}H_{38}O_2$	310.51	+		0.14
52-	Coumarin,7,8-dihydro-7-hydroxy-6-methoxy-8-oxo-	15.57	$C_{10}H_8O_5$	208.167496	+		1.30
53-	Geranyl-p-cymene	16.05	$C_{18}H_{26}$	242.3990	+		1.48
54-	2-hexadecanol	17.77	$C_{16}H_{34}O$	242.44056	+		4.44
55-	Menthol,1'-(butyn-3-one-1-yl)-,(1S,2S,5R)-	17.8	$C_{14}H_{22}O_2$	222.32		+	2.85
56-	Hexadecanoic acid, methyl ester	18.57	$C_{17}H_{34}O_2$	270.4507	+		4.26

Antioxidant activity is the total capacity of antioxidants for eliminating free radicals in the cell and in food. Antioxidants induce their effects through different mechanisms. As ascorbic acid mechanism of action is based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen and removal of molecular oxygen [22].

Polyphenols are excellent antioxidants due to a 30-40 dihydroxy group in their B ring and the galloyl ester in the C ring of flavonoids, which are also important structures in metal ion chelation [23,24]. Tocopherols antioxidant activity includes hydrogen atom transfer at the 6-hydroxyl group on the chroman ring and scavenging of singlet oxygen and other reactive species [22].

Fermentation was found to reduce flavonol glycosides part of total phenolic compounds in green tea, as a result of oxidative degradation [25]. As a result of fermentation, tea catechins were significantly reduced by the transformation to theaflavins and thearubigins, resulting in the loss of the total soluble phenolic content and antioxidative activity. We propose that increase or decrease of antioxidant activity of plant extract by fermentation may be influenced by various factors, including the microorganism species, pH, temperature, solvent, water content, fermentation time, kind of food and aerobic conditions. On the other hand, antioxidant activity of plant extract cannot be predicted on the total phenolic content alone; since synergism between polyphenolic compounds and other components may influence the measured antioxidative activity [26].

Cancer is one of the major health problems in both developed as well as developing countries that requires newer approach for its treatment, control and prevention [27]. Nowadays researchers are focusing their research towards the development of an eco friendly anti caner drug from plant sources, which may result in newer chemotherapeutic agents.

Cytotoxicity of the non-fermented and fermented plant extract to high proliferative cells as Ehrlich ascites carcinoma cells was assessed using MTT assay. Fermented *S. argel* extract showed almost two folds higher cytotoxic activity against EACC compared to the original plant. This increase in cytotoxicty might be referred to the formation of new compounds and derivatives of high antitumor and anti-proliferation activity affecting signaling pathways and activation of apoptosis and regulation of growth signaling [28].

In the present study GC-MS analysis showed that fermentation of the methanol extract elevated the number of identified compounds from 25 to 36 compounds. These new formed compounds are—propably responsible for—the improvement of the antitumor activity observed in fermented *S. argel*. These phytochemical compounds interaction and combination play a crucial role in the potential anticancer remedy [26].

Many compounds were identified in in F.*S. argel* extract found to have antiproliferation activity, of these compounds: 4-Methylcatechol was found to inhibit the growth of melanoma cells not the normal cells. Melanoma cells growth inhibition is associated with increased ROS which induce apoptosis through down regulation of pro-apoptotic Bcl2 and pro-survival pAkt [29](Payton et al., 2011). Also, p-Coumaric acid was found to exert antiproliferative activity against colon cancer cells like HT 29 and HCT 15 by inducing apoptosis [30].

Alpha-copaene is a tricyclic sesquiterpene derived from different plants. Previous studies indicated its anticarcinogenic, antioxidant, hepatoprotective, antiinflammatory [31,32], antigenotoxic and antioxidant activities [33,34].

GC/Mass also identified, alpha terpineol. This compound was reported to exhibit anti-proliferative effect on human erythroleukaemic cells [35]. α-tocopherol was also identified and previously reported to have anti-cancer micronutrient, promoting tumor dormancy, apoptosis and inhibit cell proliferation in melanoma, colon cancer and breast cancer [36] as a single agent or 2- to 3-drug combination [37].

Sinapic acid was found to possesse a wide variety of biological activities, especially as antioxidants and antibacterial activity in vitro [38]. Previous reports documented that; Sinapic acid is a potent antioxidant than ferulic acid and sometimes comparable to that of caffeic acid [39]. Sinapic acid may be a potent cytotoxicity effects on HEp-2 cancer cells, by inhibiting the growth of the cancer cells, through the apoptotic cell death and cell cycle arrest [40].

Analysis also identified the presence of ferulic acid which among its multiple pharmacological and biological effects exerts antitumor activity and anti-cell proliferative by decreased expression of cyclin D1 [41,42,27].

In addition, apigenin has been reported to modulate vascular tone. It is a polyphenolic bioflavone, a non-mutagenic chemopreventive agent [43]. Oral administration of apigenin inhibited markedly the proliferation of cancer cells in ovarian tumor, micro-metastasis in liver, lung, small intestine and stomach in different degrees through inhibiting MMP-9 expression, the migration of endothelial and tumor cells [44,45,46].

Also, Quercetin was found to be effective chemopreventive and chemotherapeutic agent for its powerful antioxidant and free-radical scavenging properties [47]. The cellular and molecular mechanisms of quercetin were mainly via cell cycle arrest accompanied by mitochondria-mediated apoptosis which inhibits cell growth and invasion/migration on SCC-25 cells *in vitro* [48].

Esculetin is a derivative of coumarin, and effective agent against human colon cancers through inhibiting Wnt–b-catenin signaling, which acts by targeting b-catenin to effectively suppress proliferation of human colon cancer cells both *in vitro* and *in vivo* [49].

Phenolic compound scopoletin was also identified and found to have antitumor, antiangiogenic activities [50]. Also, Coumarin compounds possess strong antiproliferative activity and induce apoptosis in various cancer cell lines such as A549, ACHN, H727, MCF-7, HL-60, prostate cancer, malignant melanoma, and metastatic renal cell carcinoma [51].

5. CONCLUSION

The current work could be considered the first on identification and evaluation of antitumor activity of Lactobacilli fermented *S. argel*. Fermentation process was found to improve antitumor activity via synthesis of new chemical compounds and derivatives, although total phenolics and flavonoids contents were not affected, DPPH radical scavenging activity was significantly reduced, with significant increase in the IC50 inhibitory concentration**.** That, antitumor activity of *S. argel* is relied on new compounds of high antitumor activity formed due to fermentation process and not on the antioxidant activity of the extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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