Characterization and assessment of antioxidant activity of some phenolic extracts of ginseng roots (*Panax ginseng*)

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Abstract

Despite of wide use of ginseng products in customary medication over hundreds of years, yet, the clinical medication is inconclusive with regards to its clinical credence nor is it supported by the American Food and Drug Administration (FDA) as a prescriptive medication. Ongoing researches showed that ginseng products might be contaminated with poisonous metals or other filler mixtures, and its excessive use might contribute to some unexpected side effects or untoward interaction with prescriptive medication. Phenolic compounds (phenols and flavonoids) are extracted from the roots of Chinese ginseng plant (*Panax ginseng*) using organic solvents i.e. hexane, chloroform, ethyl acetate, ethanol, and methanol. Their amounts, efficacy for binding the ferrous ion, antioxidant activity in reducing power, and their ability to bind iron over the rest of the extracts have demonstrated the elevated 2,2-diphenyl-1-picrylhydrazyl radical, ferrous ion chelating and hydroxyl radical scavenging activities. Using gas chromatography-absorption spectroscopy (GC-AS) technique, a few phenolic compounds were diagnosed e.g., 9, 10-Secocholesta-5; 7,10(19)-triene-3; 25, 26-triol, [3-beta, 5Z, 7E]; the [7R, 10R]-1,5-Endoperoxy-2-hydroper oxycarota-1,4-dien-14-al) to have the highest concentration among 10 other diagnosed compounds. The impact of antioxidant activity of a few phenolic compounds extracted from ginseng roots (*P. ginseng*) is attributed to the potency of ginseng product in inhibiting the oxidative activities of radicals in protecting the cell membrane components from damage. Further biochemical assessment are required to purify these extracts and to assess their pharmacological and therapeutic applications.

Keywords: Antioxidant activity, Gas Chromatography-Absorption Spectroscopy, flavonoids, *Panax ginseng,* phenolic compounds.

1.0. Introduction

The ginseng refers to some plants in genus *Panax* e.g. *P. notoginseng; P. ginseng*, as well as the American ginseng *P. quinquefolius*, are normally known to contain both ginsenosides and gintonin in Kim [1]. There also seems no substantial evidence that ginseng is effective for treating any medical condition nor its use is approved by the US Food and Drug Administration (FDA) as a prescriptive medication [2, 3]. Clinical trials, case reports, both *in vitro* and *in vivo* researchers found that patient risks are associated with ginseng abuse and/or misuse such as allergy, affective disorder, genital organ bleeding, cardiovascular and renal toxicity, hepatotoxicity, reproductive toxicity, gynecomastia, hypertension, and anticoagulant-ginseng interaction [4]**.** While, ginseng is commonly sold as a dietary supplement, concerns are raised about synthesized products containing toxic metals or filler staff i.e. rice or wheat or unrelated compounds [5-7]. The *Panax. ginseng*, is known to be one of the most important and commonly used plant to maintain body homeostasis as it contains many active compounds e.g. acid polysaccharides, ginsenosides phenols as well as polyethylene compounds that confer many of its pharmacological effects i.e. anti-oxidative, anti-stress effects, anticancer and enhance both immune system and liver function [8, 9]. Many scientist introduced a new pharmacological concept to the meaning of tonic effect of ginseng, resulted in raising interest and attention via explaining the basic pharmacology with adaptogen effects of ginseng [10]. These contents of *P ginseng* deem it most important orient plant to extract some valuable products and therefore to test their activity for pharmacological efficacy and consequence medical applications.

Using the most reliable techniques we hereby characterize, assess the antioxidant activity of a few phenolic compound extracts from ginseng roots (*Panax ginseng*). It is believed that our results will further explore the biochemical mechanism of action of these extracts on various human bodies and clarify their pharmacological and therapeutic impacts or/and side effects in medicine and food products.

2. Materials and methods

2.1. Roots of the ginseng plant

Roots of the ginseng plant were obtained from local Medical-herb shops in Kerkuk governorate. They were washed to remove dirt and dust, dried and followed by grinding the dry roots to obtain a homogeneous powder for subsequent uses.

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2.2. Estimation of total phenolic contents

Using the modified Folin-Ciocalteu method [11] the total phenolic content (TPC) of *P ginseng* extracts are measured. A 2 mL solution of the extracts or standard were transferred into a 10 mL volumetric flask separately. To each flask, only 1 mL of Folin-Ciocalteu reagent diluted with (1:10 [v/v] distilled water and topped up with one mL of sodium carbonate. The reaction mixture was vortexed for 15 seconds and left for 15 min. at room temperature for color development, following by incubating the reaction mixture and absorbance was recorded at 765 nm via spectrophotometer. The standard curve produced with varying concentrations of Gallic acid (R2=0.9986) was used to assess the amount of phenolic content in the sample. The TPC was expressed as percentage of Gallic acid equivalents/100mg (GAE) dry weight of plant sample.

2.3. Estimation of total flavonoid contents

Aluminum chloride ($AlCl_3$) method was used to determine total flavonoid content of *P ginseng*. Only 1 mL of (2%) $AlCl_3$ methanol solution was added into 3 mL of different dilution of standard solution of Quercetin (5, 10, 15, 20 and 25 μg/mL, respectively) or plant extract and left for 15 min. at room temperature. Followed by mixing the solution and the absorbance was recorded against a freshly prepared blank reagent at 420 nm using spectrophotometer. The standard curve produced with various concentrations of Quercetin (R2=0.999) was adopted to assess the total flavonoid content of plant sample. The TFC of plant extract was presented as percentage of Quercetin equivalents/100 mg dry weight of the sample.

2.4. Estimation of antioxidant activity

For the extracts, the Ferric thiocyanate [$Fe(SCN)_3$] method was used to measure the oxidative activity of linoleic acid ($C_{18}H_{32}O_2$) by dissolving 100 μL of linoleic acid in 4 mL of ethanol (C_2H_5OH), 8 mL of 0.05M phosphate buffer (*p*H 7.0) and 3.9 mL of distilled water. A 50 μL of sample (10, 25, 50 mg/mL) was added to 1.4 mL the above linoleic acid solution. The mixture was left in darkness at 40^0C; the accelerated oxidation of the linoleic acid was measured after 24, 48, 72 and 96 hours of thermal treatment. Using the ferric-thiocyanate method determination of oxidation degree (as peroxides formation) was performed. A 30 μL of the reaction mixture was added to 2.91 mL of 75% ethanol, 30 μL of 30% ammonium thiocyanate (NH_4SCN) and 30 μL of 0.02M ferrous chloride ($FeCl_2$) in 3.5% hydrochloric acid (HCL). The mixtures were shaken and the absorbance was measured at 500 nm according to the below equation exactly after 3 min [12].

Antioxidant activity%= (Sample absorption/Control sample absorption) x100

2.5. Reducing power

For the measurement of reducing power a spectrophotometric method was used [13]. A 2.5 mL of extracts were mixed with 2.5 mL phosphate buffer (0.2M, *p*H 6.6) and 2.5 mL of 1% Potassium ferric-cyanide [$C_6N_6FeK_3$] (10 mg/mL). The mixture was incubated at 50°C for 20 min. followed by a rapid cooling, mixed with 2.5 mL of 10% trichloroacitic acid [$C_2HCl_3O_2$] and centrifuged at 6500 rpm for 10 min. Then a 2.5 mL of supernatant was mixed with 2.5 mL distilled water followed by adding a 0.5mL (0.1%) of a ferric chloride (allowed to stand for 10 minutes). The absorbance was read spectro-photo-metrically at 700 nm. Five concentration of vitamin-E (10, 25, 50, 100 and 200 mg/mL) were used to construct calibration curve and the reducing power activity was reported as Vitamin-E equivalent/100gm dry sample.

2.6. The ability to bind ferrous ion (Fe^{2+} chelation)

Using a standard method with minor changes the ferrous ion chelating activity was evaluated [14]. The reaction was carried out using HEPES buffer (20 mM, *p*H 7.2). Various concentrations (0-120 μg/mL) of plant extract were added to 12.5 μM ferrous sulfate solution and the reaction was initiated by the addition of (75 μM) ferrozine [$C_{20}H_{12}N_4Na_2O_6S_2 \cdot xH_2O$]. The mixture was vigorously shaken, incubated for 20 min at room temperature, then the absorbance was measured at 562nm. All tests were performed six times and the EDTA was used as a positive control.

2.7. Inhibiting the oxidation of oils

The method was used to estimate the ability of ginseng extract with methanol to inhibit the auto-oxidation of olive oil which included: dissolving a gram of oil in 24 mL of chloroform-methanol mixture (2:1), adding to the extract mixture with concentrations 2, 4, 6, 8, 10 mg/gm of oil. The homogeneous mixture was then incubated at a temperature of 45°C for periods of 5, 10, 15, 20, 25 days, respectively. The peroxide was estimated accordingly throughout these experiments. The control sample was treated in the same way except for adding 1 mL of distilled water to the mixture instead of the extract. The synthetic antioxidant Butyated Hydroxy Toluene (BHT) ($C_{15}H_24O$) was used for comparison at a concentration (mg/gm of oil).

2.8. Hydrogen peroxide scavenging assay

The ability of the extract to scavenge hydrogen peroxide (H_2O_2) was determined [15]**.** Aliquot of 0.1 mL of extracts (25-400 μg/mL) was transferred into the Eppendorf tubes and their volume was made up to 0.4 mL with 50 mM phosphate buffer (*p*H 7.4) followed by the addition of 0.6 mL of H_2O_2 solution (2 mM). Reaction mixture was vortexed, after 10 min of reaction time, its absorbance was measured at 230nm. Ascorbic acid [$C_6H_8O_6$] was used as the positive control. The ability of the extracts to scavenge the H_2O_2 was calculated using the following equation:

H_2O_2 Scavenging activity percentage=[(A0-A1)/A0] ×100 (where A0=Absorbance of control and A1=Absorbance of sample).

2.9. Identification of cyclic compounds using Gas Chromatography-Mass Spectrometry (GCMS)

The cyclic compounds were identified in the extract of roots of the ginseng plant using gas chromatography-connected mass spectrometry (Zeiss, model EM10C, Germany) technique in the CAC laboratory for medical examinations in Baghdad Governorate, according to the

following separation conditions: column type (30×0.25mm id, film DP-5MS thickness 0.25 μm). The helium gas was used as an inert gas with a flow rate of 1 mL/second. The temperature of the injection and the interconnector was 280°C. The oven program was set on at an initial temperature of 100°C for a minute but was raised to 280°C by 6 initial temperatures per minute and the spectra of the curves were matched with the spectral library 08NIST.

3.0. Results and Discussion

3.1. Total content of both phenols and flavonoids

Using five different solvents the concentration of phenolic was almost 2 folds of flavonoid compounds extracted from the roots (Table-1). The lowest concentration of phenolic compounds detected was with hexane. This might be attributed to the fact that most of the extracted phenolic compounds are highly polar, while some of these compounds are non-polar and therefore can be extracted only with hexane (C_6H_{14}). However, the highest concentration of phenolic compounds reached 72 mg/gm and 50 mg/gm flavonoids were with methanol, respectively. Such differences could be attributed to the different extraction conditions and the source of the plant.

(Table-1): Concentrations of phenolic and flavonoid compounds extracted from the roots of the ginseng plant using different solvents. The difference between these two amounts was significant and ranged between *(p≤0.05) and **(p≤0.001) in comparison with the hexane.

The solvent	Phenols (mg/gm)	Flavonoids (mg/gm)
Hexane (C_6H_{14})	45	25
Chloroform ($CHCl_3$)	49	32*
Ethyl acetate ($C_4H_8O_2$)	53*	38*
Ethanol (C_2H_5OH)	63**	40*
Methanol (CH_3OH)	72**	50**

3.2. Anti-oxidant efficacy

In Table 2, the antioxidant activity of linoleic acid ($C_{18}H_{32}O_2$) for extracting from the ginseng plant assessed with different solvents were significantly lower (p≤0.01) than Tocopherol (a class of organic chemical compounds e.g. methylated phenol many of which have vitamin-E activity) and Butyated Hydroxytoluene [BHT] ($C_{15}H_{24}O$). The superiority of the methanol extracts might be attributed to their high content of both phenolic and flavonoid compounds. This may be due to a strong correlation between the concentration of extracted phenolic compounds and the antioxidant activity. Oxidation is primarily responsible for antioxidant activity, and therefore it acts as an antioxidant and as polar in nature [16, 17]. The latter is in consistent with recent findings that ginseng extract with methanol was superior over extracts of ethanol, acetone, chloroform, and hexane, as noted from the table [18, 19]**.** The industrial oxidation on all extracts is due to the purity of this antagonist which contains only one compound, while the rest of the extracts contain many ineffective compounds [20, 21]**.** Hence, for high purity these extracts may need further purification processes performance.

3.3. Reduction force

In Table 2, the ability of ginseng to reduce iron ion compared to the synthetic antioxidant BHD and the natural tocopherol is shown. The methanol extract showed a superior ability in comparison with other extracts of the ginseng plant. It is also noted that reducing power was associated with the development of the antioxidant activity which might indicate accumulation of reducing compounds necessary for the development of the antioxidant effect. Hence, these compounds are responsible for the development of the antioxidant effect of the ginseng plant and perhaps to the possibility of evaluating the antioxidant effect based on measuring the evolution of reducing power. The reducing power of phenolic compounds depends both on the number of hydroxyl groups they contained and their ability to reduce the ferric ion by donating a hydrogen atom [22]**.** This result indicates that the phenolic compounds of these extracts contain hydrogen donating compounds that are able to interact with free radicals to stabilize the products and to terminate the free radical chain reaction. The reducing power of phenolic compounds is due to the presence of reduction force that react with peroxides with the ability to reduce the ferric ion by transferring it to a hydrogen atom that turns ferrous into ferric which appears as a blue color measured at a wavelength of 700 nm [23, 24]**.**

(Table-2): Antioxidant activity and reducing power (%) efficiency of the of ginseng extracts using various solvents.

Solvent	Antioxidant capacity (%)	Reduction force(%)
Hexane (C_6H_{14})	48	34
Chloroform ($CHCl_3$)	61	38
Ethyl acetate ($C_4H_8O_2$)	65	41

Ethanol (C_2H_5OH)	72	46
Methanol (CH_3OH)	65	52
BHT ($C_{15}H_{24}O$)	85	76
Tocopherol	88	95

3.4. Ferrous ion binding

The binding material, ethylene di-amine tertra-acteic di-sodium (EDTA-2-Na) and sodium citrate demonstrated a significantly higher binding ability (Table-3). This result may indicate a partial possession of ginseng extracts to metal ion binding activity meanwhile the activity synergistically contribute to the anti-oxidative effect of Linoleic acid [25]**.** The ability of ginseng extracts to bind the ferrous ion, in comparison with EDTA and sodium citrate ($Na_3C_6H_5O_7$) is demonstrated in table 4.

Table (3): The ability of ginseng extracts to bind ferrous ions.

Solvent	Ferrous ion binding capacity (%)
Hexane (C_6H_{14})	59
Chloroform ($CHCl_3$)	48
Ethyl acetate ($C_4H_8O_2$)	41
Ethanol (C_2H_5OH)	52
Methanol (CH_3OH)	60
Citric acid ($C_6H_8O_7$)	69
EDTA	80

3.5. Inhibition of oxidation of oils

The inhibitory effect of the oxidation of a mixture appeared to be highest at a concentration of 11 mg/gm of oil (Table-4). The antioxidant compounds work on the bimolecular decomposition of the peroxides formed in oil, then prevent the formation of hydro-peroxides to give more stable products, in addition to self-oxidation adaptor action via interfering with the free radical chain reaction [21]. At concentrations 3 and 6 mg/gm of oil there has been an insignificant effect which was contributable to their inability to reduce the levels of peroxide formed. The formation might be attributed to the decrease in the concentration of antioxidant compounds at these two concentrations, while the two concentrations 9, 12mg/gm oil showed a significant effect in only the first storage periods followed by a rapid increase in the values of peroxide at these two concentrations. The latter might be attributed to the dissolution of the antioxidant compounds by the influence of temperature. The antioxidant BHT had achieved significantly ($p \leq 0.05$) higher inhibitory activity to reduce peroxide levels with the progression of the storage period. In addition to estimated saturated fatty acids at around 15% prone to oxidation, the olive oil contains a high percentage of Mono-unsaturated fatty acid estimated around 73%, [23]**.**

(Table. 4): Inhibitory effects of methanolic ginseng extracts in inhibiting olive oil at different storage periods (6-26 days) using concentrations of (3-11 mg/gm) oil at a temperature of 45°C in comparison with the antioxidant BHT (concentration 3 mg/gm oil).

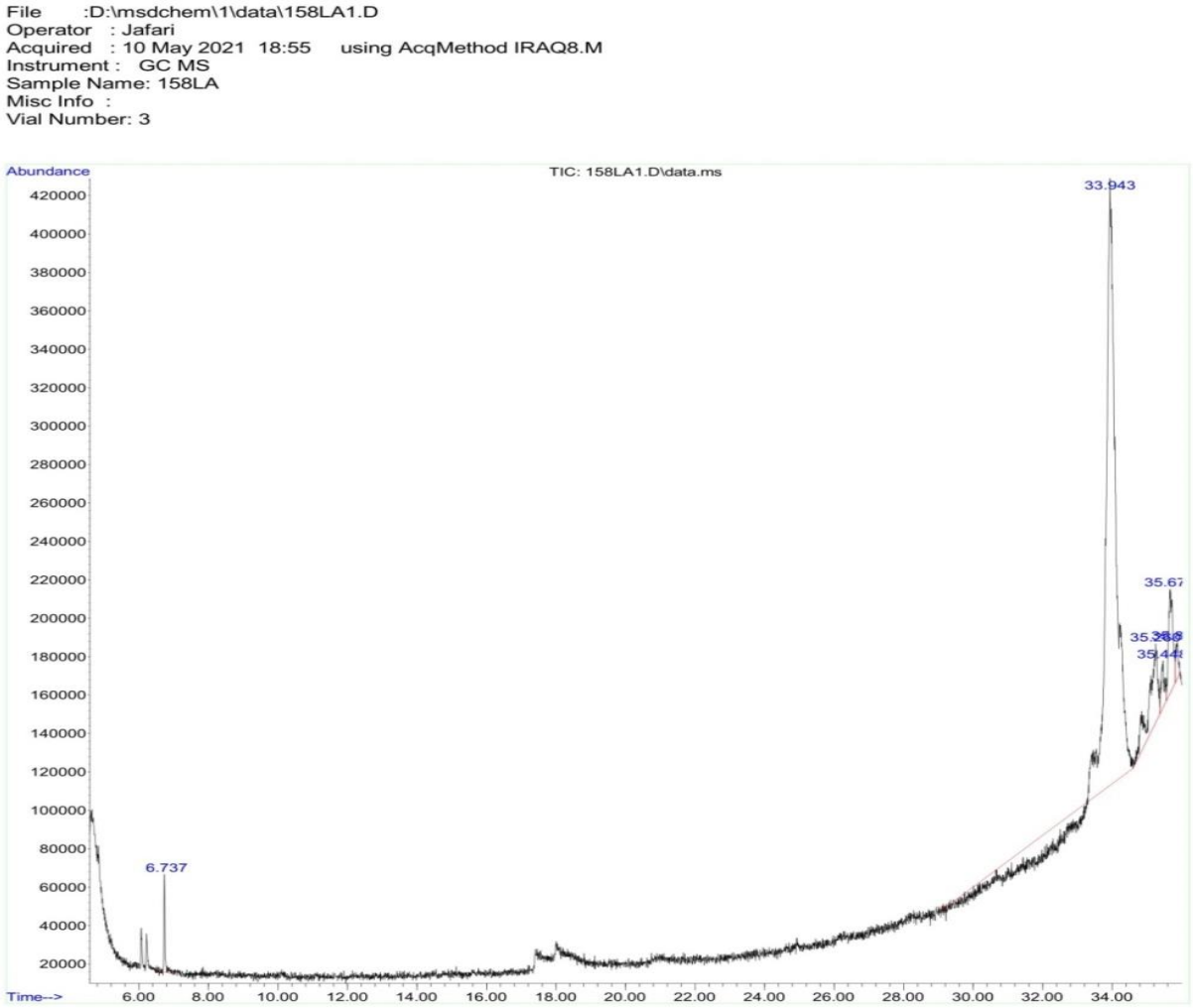
Concentrations (mg/gm of oil)	Peroxidase values (mg/Kg oil) for different periods				
	6 day	11 day	16 day	21 day	26 day
3	7	9.7	13.6	16.5	19.8
5	7.2	7.7	10.6	13.1	17.1
7	5.7	5.5	7.4	10.8	13.5
9	5.6	5.7	7.6	10.4	12.7
11	5.3	6.5	6.6	7.8	9.6
BHT (3mg/gm oil)	4.5	4.5	4.7	5.2	6.3
Control sample	7.5	11.7	15.8	18.2	20.9

3.6. Diagnosing compounds with GC-MS

Two phenolic compounds extracted with methanol using the GCMS technique i.e. (9,10-Secocholesta-5,7,10(19)-triene-3,25,26-triol, (3.beta.,5Z,7E)-) and (7R,10R)-1,5-Endoperoxy-2-hydroper oxycarota-1,4-dien-14-al) had significantly the highest concentrations, amounted to an area of 2.45% is one of the phenolic compounds that have been analyzed. This compound is classified within the group of ginsenosides antioxidant activity (Fig. 1 and Table-5). These compounds are mainly responsible for antioxidant activity of the ginseng plant. Both ethanol and methanol extracts of ginseng leaves have the potential to scavenge free radicals. Ethanol extracts demonstrated higher 2,2-diphenyl-1-picrylhydrazyl radical, ferrous ion chelating and hydroxyl radical scavenging activities. Moreover, levels of glutathione peroxidase and superoxide dismutase-like antioxidant enzymes are increased by ginseng [26]. Perhaps this potential deems ginseng so powerful natural product to cure various medical conditions which need removal of toxic radicals from tissues. The potential of ethanol extracts has recently been endorsed by some other authors Ferreira et al. [12] and the antioxidant activity of ginseng has also recently been demonstrated clinically [27].

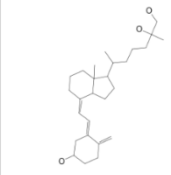
In a double-blind randomized controlled clinical trial the

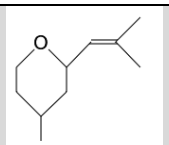
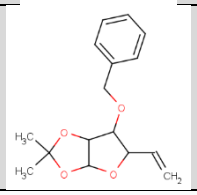
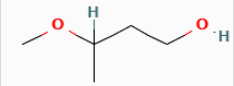
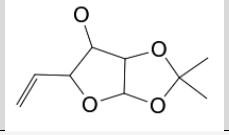
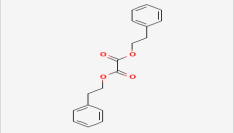
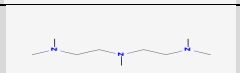
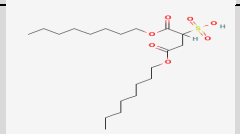
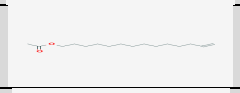
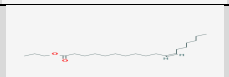
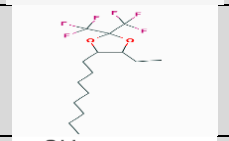
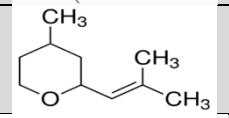
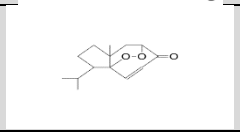
antioxidant role of administrated Korean *P. ginseng* in healthy volunteers has led to a significant decrease in the level of reactive oxygen species (ROS) and methane dicarboxylic aldehyde activity [24]. Previous studies have steadily correlated the oxidative stress to the primary and secondary causes of human disease and aging have led to multiple misconceptions about oxidative stress. Most reactive oxygen species (ROS) generated from chronic diseases can cause oxidative damage to cell membrane lipids and proteins [28]. Overproduction of ROS via abnormal stimulation inside and outside the body, and its generation in cells as a response to abnormal metabolic processes not directly contribute to the pathogenesis of a disease [27]. The impact of ginseng might therefore be to inhibit the oxidative activities of radicals and to protect the cell membrane components from damage. At present there are many industrial fat antioxidants on a commercial level i.e. Butylated Hydroxy Anisole (Butylated Hydroxy Toluene, BHD, BHA, PG Propyl gallate) [29]. Many of these properties have been confirmed, including its anti-inflammatory and antioxidant properties, anti-obesity and obesity, anti-allergic, treats blood pressure disorders. It improves memory, anti-diabetic, anti-tumor, and it is also an immune booster. Ginseng, whether roots, stems, leaves, or extracts, seems to restore the balance of immunity and secrete resistance to diseases and microbial attacks by affecting the immune system. Such reports open the research options wide for further research to confirm the positive impact of the ginseng plant for future works. In recent years, doubts have been raised about the safety of these antioxidants from a health point of view. The present research represents a new method of extraction natural resources and interprets their impact of ginseng represented by new extracts.



(Fig.1): Identification of the cyclic compounds present in the methanol extract of ginseng using GCMS.

(Table-5): Identification of various cyclic compounds present in ginseng extract using GCMS.

	Compound	Mol.weight	R. T	Area%	Similarity	Structures
1.	9,10-Secocholesta-5,7,10(19)-triene-3,25,26-triol, (3.beta.,5Z,7E)-		35.867	1.57	97	

2.	Cis-2-(1'-Isobutenyl)-4-methyl-tetrahydropyran	154.25 gm/mol.	35.45	2.45	66.14	
3.	a-D-xylo-Hex-5-enofuranose,5,6-dideoxy-1,2-O-(1-methylethylidene)-3-O-(phenyl-methyl)-	276.33	6.7	2.1	5.93	
4.	3-Methoxy-1-Butanol	104.15	6.736	2.10	12.97	
5.	Alpha.-D-Xylo-Hex-5-enofuranose,5,6-dideoxy-1,2-O-(1-methylethylidene)-	186.21 g/mol.	6.736	2.10	12.97	
6.	Oxalic acid, di(2-phenylethyl) ester	298.3	33.941	74.9	74.96	
7.	1,1,4,7,7-Pentamethyldiethylenetriamine	173.30	33.941	74.96		
8.	Succinic acid, sulfo-1,4-dioctyl ester	422.6	33.9	11.35	6.61	
9.	13-Tetradecen-1-ol acetate	254.41	35.261	11.5	88.2	
10.	n-Propyl 11-octadecenoate	324.5	35.8	1.57	65	
11.	4-Ethyl-5-octyl-2,2-bis(trifluoromethyl)-1,3-dioxolane	350.34	35.867	1.57	60	
12.	Tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-2H-pyran	154.25	35.6	7.5	69	
13.	(7R,10R)-1,5-Endoperoxy-2-hydroperoxycarota-1,4-dien-14-al		35.4	2.45	43	

4.0. Conclusion

Using GC-MS to produce more purified extracts of Ginseng. The impact of these products could be attributed to inhibiting the oxidative activities of radicals by terminating the free radical chain reaction and to protect the cell membrane components from damage.

5.0. Acknowledgement

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