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Nutrients and Nutraceuticals Content and *In Vitro* Biological Activities of Formulae from Plant Parts Commonly Spread in Egyptian Markets

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Abstract The current study aims to determine the nutrients and nutraceuticals content and *in vitro* biological activities of suggested poly-plant parts formulae. Formula I contains milk thistle, black seeds, tomato pomace, ginseng, cinnamon, eucalyptus, marjoram, molokhia, sweet fennel, black pepper, turmeric and gum arabic, while Formula II contains milk thistle, spearmint leaves, marjoram leaves, sweet fennel, lemon peels, potato peels, orange peels, onion skin, apricot kernels, turmeric, gum arabic. Data of the nutrients composition of the suggested formulae powder indicated that carbohydrates (49.87 to 51.50 $g.100g^{-1}$) was the largest compound followed by crude fat (17.45 to 18.32 $g.100g^{-1}$), total protein (15.08 to 16.23 $g.100g^{-1}$), ash (10.06 to 12.86 $g.100g^{-1}$) and crude fiber (3.87 to 4.76 $g.100g^{-1}$). Also, suggested formulae powder is rich in different estimated elements (Na, Fe, Zn, Mn, Ca, Cu, P, Mg, K and Se) and vitamins (A, B2, B6, B9, C and E). Furthermore, nutraceuticals (bioactive compounds) content of suggested formulae powder indicated that phenolics were the largest compound followed by polysaccharides, terpenoids, flavonoids, saponin, triterpenoids, oxalate, kaempherol and lycopene. Also, the content of these compounds except terpenoids, triterpenoids, and oxalate are higher in formula I than formula II. The different extracts of the suggested formulae samples particularly ethanolic and methanolic also recorded several strong biological activities including antioxidant and scavenging activities, inhibition of low density lipoprotein oxidation (anti-atherosclerotic) and as antibacterial and antifungal activitis.. Such important biological effects could play important roles in strategies to combat/ prevent/treat many diseases, especially those for which oxidative stress is one of the mechanisms for its occurrence i.e. diabetes, cancer, atherosclerosis, cardiovascular diseases etc. Therefore, the present study recommended like of these formulae powder and/or extracts to be included in our daily diets, drinks, food supplementation and pharmaceuticals.

Keywords: *chemical composition, bioactive compounds, antioxidant activity, scavenging activity, inhibition of LDL peroxidation, antibacterial*

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1. Introduction

Currently, many countries in the world including Egypt, are suffering from a large food gap, which means a disproportion between the quantities of necessary food and the number of inhabitants [1]. This food gap leads the state to import food from abroad to compensate for this shortage in order to avoid the emergence of diseases of under/malnutrition among some different segments of society. One of the proposed solutions in this regard, which has gained great popularity locally and globally, is to search for new sources of food, especially plant parts, due to their large number and diversity of sources, their presence in various climatic conditions, and their important nutrient groups such as protein, high-quality fats, vitamins and minerals [2,3,4,5] [6,7,8,9,10]. On the other hand, they are rich in different groups of bioactive compounds with important biological activities, which play a major role as natural sources of medicine instead of expensive synthetic chemical treatments and medicines, in addition to the health complications and serious side effects that result from their use [11,12,13] [14,15,16] [17,18,19] [20,21,22]. All of these factors and more make plant parts an affordable source of nutritional ingredients, as well as a promising source of pharmaceutical compounds. In light of the above, and taking into account some economic and environmental aspects, the current study was designed to benefit from plant parts produced during food manufacturing processes as by-products or waste, as well as some parts of medicinal and aromatic plants by making combinations/formulae of them that have many nutritional and medicinal benefits.

Onion skin and potato peel are producing in large quantities in food-processing plants. The major by-products resulting from industrial peeling of onion (*Allium cepa* L.) bulbs are brown skin, the outer two fleshy leaves and the top and bottom bulbs. The outer dry layers of onion bulbs (Onion skin), which are not edible and removed before processing, have been shown to contain a wide spectrum of polyphenolic components [23]. Also, it is a source of flavour components and fiber compounds and particularly rich in flavonoids including quercetin glycosides [24,25]. Since quercetin from onions and their skins is rapidly absorbed and slowly eliminated, it could contribute significantly to antioxidant defense system [26]. Also, several studies have been established for using the onion skin in different food processing applications include pectin production, pigments extraction, natural antioxidants and anticarcinogenic, vingare production, biogas productions, natural fertilizers, protection of ultraviolet adverse effects etc [27-36].

Potato is the largest vegetable crop worldwide, amounting to approximately 320 million metric tons annually [27]. Producing of potato (*Solanum tuberosum*, L.) products mainly chips, French fries, and powder has presented a steady increase during the last decades, exceeding considerably the amount of the vegetable consumed as fresh. Solid waste generated during processing consists mostly of potato peels and amounts to 10% depending on the procedure applied. Some investigations suggested the use of water extracts from potato processing waste for the recovery of antioxidants [37]. Others reported that potato skin due to containing compounds mainly phenolics with highly antioxidant properties, can play potential roles in several food technology and therapeutic nutratio applications [17] [35] [38,39] [40,41]

Marjoram, (*Origanum marjorana* L., Family *Labiatae* is a perennial herb, cultivated around the world [42]. Quantitative composition of the essential oil, volatile compounds, bioactive compounds and the antioxidant activity of marjoram were reported by [43,44] [45,46].

Fennel (*Foeniculum vulgare*) is a plant species in the genus *Foeniculum* and a member of the family *Apiaceae* (formerly the *Umbelliferae*). It is a widely distributed plant in most tropical and subtropical countries and has long been used in folk medicines to treat obstruction of the liver, spleen and gall bladder and for digestive complaints such as colic, indigestion, nausea and flatulence. In recent years the interest in this plant has increased considerably with substantial progress on its chemical and pharmacological properties [47]. Several compounds including *trans*-anethole, estragole, fenchone and polyphenolics were isolated from this plant and some of these interact with potential mechanisms of the body including antioxidant, antimicrobial and inhibition of lipid peroxidation [48].

Black seed *(Nigella sativa*) seed is variously called fennel flower, nutmeg flower, Roman coriander, black seed or black caraway. It is used as part of the spice mixture in food processing, most recognizably in bread. Several studies also indicated that black seed contains different classes of bioactive compounds so used as antioxidants, immunostimulant and antibacterial drugs [49].

Cinnamon (*Cinnamomun verum*) is a famous beverage a long history and is one of the world's most. It was imported to Egypt from China as early as 2000 BC. Cinnamon is principally employed in cookery as a and flavouring material, being largely used in the preparation of some desserts, chocolate, spicy candies and liqueurs, and meat products [50]. It contains condensed tanins, oil, coumarins, cinnamaldehyde and flavonoids which have been shown potent antioxidant and anticarcinogenic activities [51,52]. Extracts of cinnamon have also been shown to have antioxidant effects in part through activating antioxidant enzymes, prevent free radical formation, remove radicals before damage can occur, repair oxidative damage, eliminate damaged molecules, inhibit lipid peroxidation [53,54][55,56,57].

Milk thistle *Silybum marianum* L.) belongs to the *Asteraceae* family is an annual/biennial plant native of Mediterranean area and now growing and cultivated worldwide including Egypt [58,59]. *Silybum marianum* has been used for centuries in medicine, mainly to treat kidney, spleen, liver, and gallbladder diseases [60]. Plant fruits/seeds contain a mixture of flavonolignans collectively known as silymarin. Traditional *Silybum marianum* extract is made from the seeds, which contain approximately 4–6% silymarin [61].The extract consists of about 65–80% silymarin and 20–35% fatty acids, including linoleic acid. Regarding the beneficial properties of *S. marianum* and main constituent, silymarin in the treatment of dyslipidemia, diabetes, coronary heart disease and obesity [62,63,64]. Also, different pharmacological functions of silymarin were reported in liver diseases: antioxidant, antifibrotic regenerative (stimulate hepatic regeneration, choleretic, hepatoprotective, immunostimulating, and anti-inflammatory) [65,66,67].

Turmeric *(Curcuma longa* L.) belongs to the *Zingiberaceae* family along with the other noteworthy members like ginger, cardamom and galangal. Plants are gathered each year for their rhizomes, some for propagation in the following season and some for consumption [68]. Turmeric contains a wide variety of bioactive compounds including curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmeronols [69]. Also, it contains 2-8% curcumin [70]. Since the time of 1900 BC numerous therapeutic activities have been assigned to turmeric for a wide variety of diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders, and inhibition of tumors formation and promotion as cancer initiation [71,72,73,74].

Tomato (*Lycopersicon esculentum* L.) juice is the most important vegetable juice with respect to per capita consumption. About 3–7% of the raw material is lost as waste during tomato juice pressing [75]. Tomato pomace consists of the dried and crushed skins and seeds of the fruit [76]. Lycopene is the principal carotenoid causing the characteristic red hue of tomatoes. Most of the lycopene is associated with the water-insoluble fraction and the skin [77]. Therefore, skin extracts are especially rich in lycopene. Baysal et al., [78] clearly stated that a large quantity of carotenoids is lost as waste in tomato processing. Lycopene is associated with antioxidant status, gap-junction formation, inhibition of cholesterol synthesis and prevent cancer and heart diseases [79,80]. Also, the antioxidant defense system of other bioactive compounds found in tomato pomace includes vitamins (C, E), minerals (selenium, copper), phytonutrients (β-carotene, lutein), and biological products (bilirubin, coenzyme Q10) that protect tissues from oxidative damage [34].

Eucalyptus (*Eucalyptus globules;* Family: *Myrtaceae)* is a large tree with smooth, pale or ash-grey bark, becoming 3 to 20 m high. The leaves of young trees and the first leaves of young shoots are opposite, sessile, oval-oblong, with a cordate base, farinaceous-glaucous, and the older leaves are dangling, spirally arranged, lanceolate-falcate and up to 30 cm long [81]. The leaves contain 1 to 3 % volatile oil, depending on provenience [82]. The native oil contains 45 to 75% 1,8-cineole. Additionally, myrtenole, a-pinene, p-pinene, pinocarvone, y-terpinene, a-phellandrene, several aldehydes and the sesquiterpenes alloaromadendrene, aromadendrene, globulole, camphor and a-grujunene [83]. Eucalyptus oil (EO) and its major component, 1,8-cineole, have antimicrobial effects against many bacteria, viruses, and fungi. Surprisingly for an antimicrobial substance, there are also immune-stimulatory, anti-inflammatory, antioxidant, analgesic, and spasmolytic effects. Of the white blood cells, monocytes and macrophages are most affected, especially with increased phagocytic activity [84].

Molokhia *(Corchorus olitorius*) is a leafy, green, summer vegetable. A traditional dish in Egypt and Sudan, some people believe it originated among Egyptian during the time of the Pharaohs. Molokhia contains phenolics, tannins, saponins and carotenoids [8,14] [22,85]. Such compounds showed many biological properties including antidiabetic, anti-obesity, anticarcinogenic and sedative [8,14] [22,85,86].

Ginseng (*Panax ginseng*, Family: *Araliaceae*) is an herb used in traditional medicine for thousands of years [87]. It contains multiple active constituents including ginsenosides, polysaccharides, peptides, phytosterols, polyacetylenes, polyacetylenic alcohols, flavonoids, volatile compounds and fatty acids that have been shown to have different effects on carbohydrate and lipid metabolism, cognition and angiogenesis as well as on the function of neuroendocrine, immune, cardiovascular and central nervous systems [88,89,90]. Also, ginseng exhibit prevention of free radical damage and support of cardiovascular health, enhanced immunity and mental capacity [55] [91,92] [93,94].

Apricot *(Prunus armeniaca* L.; Family: *Rosaceae*) is a fruit or the tree that bears the fruit of several species in the genus *Prunus*. Fruits kernels contain alkaloids and phenolic compounds [95]. Such compounds showed antioxidant, antitumorigenic and anticarcinogenic in experimental animals [95]. Gum Arabic (GA) or Acacia gum is an edible biopolymer obtained as exudates of mature trees of *Acacia senegal* and *Acacia seyal* which grow principally in the African region of Sahe in Sudan. The exudate is a non-viscous liquid, rich in soluble fibers, and its emanation from the stems and branches usually occurs under stress conditions such as drought, poor soil fertility, and injury [96]. Regarding the GA structure, it is a neutral or slightly acidic salt of a complex polysaccharide composed of galactose, arabinose, rhamnose, glucuronic acid, 4-*O*-methylglucuronic acid, calcium, magnesium, and potassium [97]. In the last two decades, the effective biological roles of GA has been confirmed including reduction in plasma cholesterol level [98], anticarcinogenic effect [99] and antioxidant effect [100,101,102,103] with a protective role against hepatic and cardiac toxicities. In addition to that, it has been claimed that GA alleviates effects of chronic renal failure [104,105,106]. GA (dietary fiber> 80%), is associated with beneficial effects on fat metabolism [107,108]. It can serve to reduce obesity and therefore prevent associated complications in humans including coronary heart disease, stroke and diabetes. Although all previous studies and others dealt with many of the biological effects of the aforementioned plant parts and herbs, most, if not all, of these studies that were conducted in this regard were tested on these substances individually. For that reason, there was a need to bring some of these materials together in the form of combinations, in the hope that this would lead to positive interfering effects that would result in bringing together the biological activities of these plant parts and increasing their concentration.

Therefore, the current study aims to determine the nutrients and nutraceuticals content and *in vitro* biological activities of suggested poly-plant parts formulae. Formula I contains milk thistle, black seeds, tomato pomace, ginseng, cinnamon, eucalyptus, marjoram, molokhia, sweet fennel, black pepper, turmeric and gum arabic, while Formula II contains milk thistle, spearmint leaves, marjoram leaves, sweet fennel, lemon peels, potato peels, orange peels, onion skin, apricot kernels, turmeric, gum arabic.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Parts

Plant parts used in formulae preparation were purchased from the herbs merchandize, local markets, Egypt. Plant parts include: Marjoram "*Origanum majorana* " leaves, Molokheiya "*Corchorus capsularis* Linn" leaves, Sweet fennel "*Foeniculum vulgare* ", Ginseng "*Panax ginseng* ", Black seeds "*Nigella sativa* L. ", Eucalyptus "*Eucalyptus globules* " leaves, Cinnamon "*Cinnamomum verum* ", Spearmint "*Mentha spicata* " leaves Lemon peels "*Citrus limon*", Orange "*Citrus sinensis*" peels, turmeric *(Curcuma longa* L.*)* rhizomes were purchased from Agricultural Seeds, Spices and Medicinal Plants Company (Harraz), El-Darb El-Ahmar, Cairo Governorate, Egypt. Onion skin "*Allium Cepa* L" and tomato (*Lycopersicon esculentum* L.) pomace were obtained as a donation from New Bani Suef Company for Prsevation, Dehydration and Industratzation of Vegetables, Bani Suef El-Goudida City, Nile east, Bani Suef, Egypt. Potato peels "*Solanum tuberosum*" was obtained as a donation from Safco Company, El-Nigella Village, Beheira Governorate, Egypt. Apricot "*Prunus armeniaca*" kernels were obtained from Amar Village, Al-Qalyubia Governorate, Egypt. Milk thistle (*Silybum marianum* L.), dried fruits of wild plant populations growing in public irrigation canals were obtained with special arrangements from Mit Ghoarb Village of, Sinbillawin, Dakahlia Governorate, Egypt.Gum arabic "*Acacia senegal* L." was obtained from the SAVANNA Companies Group

(Processing Gums, Juices and Confectionery), Khartoum, Sudan (Specification: appearance colouroff white, appearance form- powder, purity, 98.34±0.67%). Taxonomic confirmation of the all plant parts were achieved by the plant taxonomy scientist in the Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt.

2.1.2. Chemicals

Bioactive compounds standard [gallic acid (GA), catechine (CA), lycopene, kaempherol, catechin and oxalic acid], represent vitamins standard (β-carotene, ascorbic acid, α-tocopherol, riboflavin, pyridoxine and folate), butylated hydroxytoluene (BHT), DPPH (2,2-diphenyl-1-picrylhydrazyl), AAPH [2,2′-Azobis(2-methylpropionamidine) dihydrochloride], $CuSO_4$ and dimethyle sulfoxide (DMSO) were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals (except as mentioned elsewhere), reagents and solvents were of analytical grade were purchased from El-Ghomhorya Company for Trading Drug, Chemicals and Medical Instruments, Cairo, Egypt.

2.1.3. Machines

Throughout this study absorbance (Abs) for different assays were measured using UV-160A; Shimadzu Corporation, Kyoto, Japan. Also, atomic absorption spectrophotometer, type Perkin – Elmer, Model 2380, Waltham, MA, USA was used for mineral determination. Furthermore, SP Thermo Separation Products Liquid Chromatography (Thermo Separation products, San Jose, CA, USA) was used with a Consta Metvic 4100 pump, a Spectra Series AS100, Spectra System UV 1000 UV/Visible Spectrophotometer Detector, Spectra System FL 3000 and a PC 1000 system software. The columns used (Alltech, Deerfield, IL, USA) were a reversed-phase water Adsorbosil C_{18} (5 µM, 100 mm × 4.6 mm I.d.) for water-soluble vitamins; and normal Ultrasphere Si (5 µM, 250 mm × 4.6 mm I.d.) for analysis of fat-soluble vitamins.

2.1.4. Biological Model

Adult male albino rats (160 g per each) were obtained by special arrangement from Helwan Station, Ministry of Health and Population, Cairo, Egypt. Rats were housed, maintained, kept under normal healthy conditions in accordance with the National Research Council's Institute of Laboratory Animal Resources, Commission on Life Sciences rules [109]. The basic diet for rats feeding protocol and the components of the salt mixture and vitamins that were used were prepared in accordance with AIN [110].

2.2. Methods

2.2.1. Preparation of Plant Parts Powder

All plant parts used in formulae preparation were manually sorted to remove foreign materials and broken and damaged parts. The parts were dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at 45-65 ^{0}C (According to the type of plant part))until arriving by the moisture in the final product to about 8%. The dried parts were ground into a fine powder in high mixer speed (Toshiba ElAraby, ElAraby Co., Benha, Egypt). The material that passed through an 80 mesh sieve was retained for the formulae preparation and analysis.

2.2.2. Preparation of the Suggested Formulae

The suggested formulae were prepared by weighing the components of each blend separately according to mentioned in Table 1, then mixing them together and homogenizing them using a high-speed blender (Toshiba ElAraby, ElAraby Co., Benha, Egypt). The resulting formulae were placed in glass jars and stored in refrigerators at 4°C until used for study purposes.

Table 1. Recipes of the proposed formulae

Formula I		Formula II	
Plant part	Percentage (%)	Plant part	Percentage (%)
Milk thistle	20.0	Milk thistle	20.0
Black seeds	4.0	Spearmint leaves	9.0
Tomato pomace	8.5	Marjoram leaves	7.5
Ginseng	8.5	Sweet fennel	9.5
Cinnamon	8.5	Lemon peels	8.0
Eucalyptus	7.0	Potato peels	8.0
Marjoram	9.5	Orange peels	8.5
Molokhia	11.0	Onion skin	8.5
Sweet fennel	7.5	Apricot kernels	7.0
Black pepper	1.5	Turmeric	4.0
Turmeric	4.0	Gum arabic	10.0
Gum arabic	10.0	Total	100
Total	100.0		

2.2.3. Preparation of the Suggested Formulae Extracts

According to the method mentioned by Gharib et al., [20], 20 gram of each formula dried powder were extracted with 80% aqueous solvents i.e. methanol and ethanol as well as water (180 ml) on an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for 120 min at 70^{0}C for the organic solvents and 120 min at 100^{0}C for the water. The mixture was subsequently filtered (Whatman No. 5) on a Buchner funnel. The residual solvents were removed under reduced pressure at 55°C for water and 40^{0}C for organic solvents using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). The obtained extracts were stored at 4^{0}C before use.

2.2.4. Chemical Analysis of the Suggested Formulae

Suggested formulae samples were analyzed for proximate chemical composition including moisture, protein (T.N. × 6.25, micro - kjeldahl method using semiautomatic apparatus, Velp company, Italy), fat (soxhelt miautomatic apparatus Velp company, Italy, petroleum ether solvent), ash, fiber and dietary fiber contents were determined using the methods described in the A.O.A.C. [111]. Carbohydrates calculated by differences: Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber).

2.2.5. Nutritional Value Determination of the Suggested Formulae

2.2.5.1. Total Energy Value

Total energy (Kcal/100 g) of the suggested formula powder samples was calculated according to Insel *et al*, (2002) using the following equation: Total energy value (Kcal/100 g) = 4 (Protein % + carbohydrates %) + 9 (Fat %)

2.2.5.2. Satisfaction of the Daily Needs of Adult Man (25-50 Year Old) in Protein

Grams consumed (G.D.R. g) of the suggested formulae powder (dry weight basis) to cover the daily requirements of adult man (63 g) in protein was calculated using the RDA values [112]. Percent satisfaction of the daily requirement of adult man in protein (P.S., %) when consuming the possibly commonly used portions in Egypt i.e. one bag (100 g weight), was also calculated.

2.2.5.3. Satisfaction of the Daily Requirements of Adult Man (25-50 Year Old) in Energy

Grams consumed of the suggested formulae powder (dry weight basis) to cover the daily requirements of man in energy (G.D.R. g) were calculated using the RDA (Recommended dietary allowances) which are 2900 Kcal /day for man as given by RDA [112]. The percent satisfaction (P.S., %) of the daily needs of adult man (25 -50 year old, 79 Kg weight and 176 cm height) in energy upon consumption the commonly used portion at homes in Egypt, i.e. i.e. one bag (100 g weight), was also calculated.

2.2.6. Determination of Minerals Content

Minerals content of the suggested formula powder samples were determined according to the method mentioned by Singh et al., [113]. In brief, 0.5 g of defatted sample were transferred into a digested glass tube of Kjeldahl digestion unit and 6 ml of tri-acids mixture (containing nitric acid: perchloric acid : sulfuric acid in the ratio of 20 : 4 : 1 v/v respectively) were added to each tube. The tubes content were digested gradually as follow, 30 min at 70°C; 30 min at 180 ^{0}C and 30 min at 220°C. After digestion, the mixture was cooled, dissolved in distilled water, and the volume was increased to 50 ml in volumetric beaker. After filtration in ashless filter paper, aliquots were analyzed for minerals (K, Na, Mg, Zn, Fe, Cu, Mn, Ca, Se and P) using of atomic absorption spectrophotometer.

2.2.7. Vitamins Determination

Fat soluble vitamins (A and E) were extracted from the suggested formula powder samples according to the methods described by Epler et al., [114] and Hung et al., [115] while water soluble vitamins (B and C) according to Moeslinger et al., [116], and analyzed by HPLC techniques. Under the chromatographic conditions used in those methods, mean values ±SD of vitamins A, C, B_2, B_6, B_9 and E recoveries were 89.54± 1.79, 87.11 ±0.98, 84.90± 1.84, 86.73±1.12, 89.34±1.54%, and 90.16±0.94%, respectively.

2.2.8. Bioactive Compounds Determination

Total phenolics in the suggested formulae extracts were determined using Folin-Ciocalteu reagent according to Singleton and Rossi [117] and Wolfe et al., [118]. Results are expressed as gallic acid and equivalents (GAE). Lycopene was determined such as described by Gordon and Diane [119] and was expressed as µg/g of dry extract. Total flavonoids contents were estimated using colorimetric assay described by Zhishen et al., [120] and expressed as catechin equivalent, CAE (standard curve equation: y = 0.0003x - 0.0117, r^2 = 0.9827), mg of CA/g of dry extract. Total polysaccharides were extracted and measured according to the method of Vazirian et al., [121]. Starch was used as a standard and the results were expressed as mg of starch equivalents per g of dw. Total terpenoids were extracted and measured according to the method of Ghorai et al., Ghorai et al., [122]. Triterpenoids were extracted and measured according to the method of Schneider et al., [123] and was expressed as mg ursolic acid/100 g of dry extract. Saponin content was determined according to the method of Fenwick et al., [124] Gallic acid was used as a standard to establish the standard curve, from which the saponin content of sample was determined. Oxalate was determined such as described by Oke [125]. Kaempherol was measured according to the method mentioned in Fouda et al., [126].

2.2.9. Antioxidant Activity Determination

2.2.9.1. Antioxidant Activity

Antioxidant activity (AA) of **the suggested formulae** extracts and standards (α-tocopherol and BHT) was determined according to the β-carotene bleaching (BCB) assay following a modification of the procedure described by Marco [127]. The AA was all calculated as percent inhibition (bleaching rates of *β*-carotene in reactant tested formulae extracts) relative to control (bleaching rates of *β*-carotene in reactant mixture of without tested formulae extracts) such as described by Al-Saikhan [128].

2.2.9.2. DPPH Radical Scavenging Assay

Free radical scavenging ability of the suggested formulae extracts was detected by DPPH radical scavenging assay as mentioned by Desmarchelier et al., [129]. In brief, a solution was prepared, and 2.4 mL of DPPH (0.1 mM in MeOH) was mixed with 1.6 mL of the tested formulae at different concentrations (12.5–150 µg/mL). The reaction mixture was mixed and left in the dark at room temperature for 30 min. The Abs of the mixture was measured spectrophotometrically at 517 nm. BHT was used as reference. Percentage of DPPH radical scavenging activity was calculated by the following equation: DPPH radical scavenging activity (%) = [(A0−A1)/A0]×100. where A_0, Abs of the control, and A_1, Abs of the BA / BHT. Then inhibition (%) was plotted against concentration, and IC_{50} was calculated from the graph.

2.2.9.3. Inhibition of Low Density Lipoprotein (LDL) Oxidation

According to the method of Princen et al., [130], inhibition of LDL oxidation was determined in the suggested formulae extracts. In brief, serum of adult male white albino rat, Sprague Dawley strain, was collected and diluted by phosphate buffer (50 mM, pH 7.4) to the concentration of 0.6%. Amounts of 5.0 ml diluted serum were mixed with 10 µl DMSO or 10 µl DMSO containing various concentrations of the tested extracts. A 20 µl of $CuSO_4$ solution (2.5 mM) was added to initiate the reaction and the Abs at 234 nm was recorded then was taken every 20 min thereafter for 140 min at room temperature. The final results were expressed by calculation the net area under the curve.

2.2.9.4. Antibacterial and Antifungal Tests

Escherichia coli, *Staphylococcus aureus* and *Candida albicans* (from the collection of the Microbiology Department, Faculty of Agriculture, Damietta University, Damietta, Egypt) were used as test microorganisms. Antibacterial and antifungal activities for the suggested formulae extracts were elucidated by the agar cup methods described by Spooner et al., [131].

2.3. Statistical Analysis

All tests/measurements were done in triplicates and presented as mean± standard deviations (SD). Statistical analysis was performed using Student *t*-test and MINITAB 12 computer program (Minitab Inc., State College, PA).

3. Results and Discussion

3.1. Proximate Composition and Nutritional Evaluation of the Suggested Formulae

Proximate composition of the suggested formulae powder is presented in Table 2. From such data it could be noticed that carbohydrates (49.87 to 51.50 $g.100g^{-1}$) was the largest compound followed by crude fat (17.45 to 18.32 $g.100g^{-1}$), total protein (15.08 to 16.23 $g.100g^{-1}$), ash (10.06 to 12.86 $g.100g^{-1}$) and crude fiber (3.87 to 4.76 $g.100g^{-1}$). These results show that the suggested formulae are close to each other with regard to the components indicating the chemical composition. These data are partly consistent with some formulae (Formula I contains black seeds, ginseng, cinnamon, eucalyptus, marjoram, molokhia and sweet fennel, and Formula II contains black seeds, spearmint leaves, marjoram leaves, sweet fennel, lemon peels, potato peels, orange peels, onion skin, apricot kernels and gum arabic) proposed for improving carbon tetrachloride-induced liver disorders in rats [132,133]. The results of the current study also partially match some of the formulae studied by El-Safty, [14] for producing food supplements for various therapeutic purposes including sedative (Azzcalm) contains spearmint leaves, chamomile flowers, anise seeds, lemon peels, orange peels, potato peels and gum arabic, diet (Azzdiet) contains katona seeds, moloukhia leaves, fenugreek seeds, cinnamon, ginger, maramia, cinameki, onion skin and gum arabic, and strengthen immunity (Azzimune) contains black seeds, spearmint leaves, marjoram leaves, sweet fennel, lemon peels, potato peels, orange peels, onion skin, apricot kernels and gum arabic. All of these previous studies, along with others, concluded that the variation observed in the chemical composition of the suggested formulae could be mainly due to the nutritional or medicinal purpose of preparing the formula and therefore the difference in the plant parts that make it up, the proportions of each component, and the technological conditions surrounding the preparation and mixing of the components.

On the other side, the nutritional evaluation of the suggested formulae powder is shown in Table 2. From such date it could be noticed that the total energy were 427.97 and 424.68 kcal/100g, G.D.R. (g) for protein (63 g) were 388.17 and 417.77 g, G.D.R. (g) for energy (2900 Kcal) were 677.62 and 682.87 Kcal,, P.S./ 100 g for protein (63g) were 25.76 and 23.94 % and P.S./100 g for energy (2900 Kcal) were 14.76 and 14.64% for Formulae I and II, respectively. The nutritional evaluation reported was partially accordance with some formulae proposed for improving carbon tetrachloride-induced liver disorders in rats [132,133]. In general, the results of the chemical analysis and nutritional evaluation of the suggested formulae also showed some important evidence, namely, the low moisture content recorded in formulae is an indicator that may not support the growth of microorganisms and thus keep it without spoilage i.e. microbial growth for a long period of time. Also, the high fat content of the suggested formulae indicates that it could be a potential source of oil for different nutritional and therapeutic purpose but such a matter requires many future studies. Furthermore, the ash and protein contents of suggested formulae show that they are better sources of dietary minerals and protein eespecially when compared to the formulae of some other studies.The same behavior was recorded for carbohydrates content of the formulae. Therefore, the richness of these combinations in most nutritional components (proteins, fats, minerals, fibers and carbohydrates) adds to them an important nutritional characteristic in addition to their functional properties, which is their ability to meet the recommended dietary values to a large extent for children and adults.

Table 2. Proximate composition and nutritional evaluation of the suggested formulae

Component	Formula I		Formula II	
	Range	Mean ±SD	Range	Mean ±SD
Chemical composition:				
Moisture	7.98- 9.24	8.63 ± 0.67 ^{a}	8.80 - 9.99	9.48 ± 0.49 ^{a}
Dry matter	89.45 - 91.99	91.37 ± 0.54 ^{a}	89.04 -10.21	90.52 ± 0.66 ^{a}
Total protein (g/100g)	15.22- 17.98	16.23 ± 1.23 ^{a}	14.03 - 15.88	15.08 ± 0.99 ^{b}
Crude fat (g/100g)	15.24 - 18.99	17.45 ± 1.76 ^{a}	17.09 - 20.05	18.32 ± 1.09 ^{a}
Ash (g/100g)	9.75 - 10.73	10.06 ± 0.58 ^{b}	12.05 - 13.61	12.86 ± 0.44 ^{a}
Crude Fiber (g/100g)	3.81 - 5.87	4.76 ± 1.10 ^{a}	2.69 - 4.49	3.87 ± 0.97 ^{b}
Carbohydrate (g/100g)	49.21-53.98	51.50 ± 1.59 ^{a}	48.12 – 50.91	49.87 ± 1.14 ^{a}
Nutritional evaluation:				
Energy (Kcal/100g)	410.67 – 423.15	427.97 ± 6.02 ^{a}	418.09 – 431.06	424.68 ± 5.78 ^{a}
G.D.R. (g) for protein (63 g)	381.67- 401.67	388.17 ±6.14 ^{a}	408.12 – 426.76	417.77 ± 7.09 ^{a}

Component	Formula I		Formula II	
	Range	Mean ±SD	Range	Mean ±SD
G.D.R. (g) for energy (2900 Kcal)	969.56- 691.23	677.62 ± 9.56 [a]	675.09 – 691.78	682.87 ± 8.17 [a]
P.S./100 g (%) for protein (63g)	24.09- 26.94	25.76 ± 1.14 [a]	22.43 – 24.15	23.94 ± 0.99 [a]
P.S./100 g (%) For energy (63g)	13.03 – 15.67	14.76 ± 1.09 [a]	13.32 – 16.02	14.64 ± 1.11 [a]

Each value represents the mean of four replicates ±SD. Means in the same raw with different superscript letters were significantly different at $p \leq 0.05$. Moisture and dry matter were presented based on air-dried weight, others were presented based on dry weight (DW). Each value represents the mean of four replicates ±SD. G.D.R. (g), grams consumed of the suggested formulae powder (dry weight basis) to cover the daily requirements of man in protein or energy; P.S. (%), the percent satisfaction of the daily needs of adult man (25 -50 year old, 79 Kg weight and 176 cm height) in protein or energy upon consumption the commonly used portion at homes in Egypt, i.e. i.e. one bag (100 g weight).

3.2. Minerals Content of the Suggested Formulae

Data in Table 3 indicated the minerals content of the suggested formulae powder. Such data indicated that the formulae are rich in different estimated elements. With the exception of Zn, formula II recorded higher values for the rest of all estimated elements than formula I. The trend of the abundance of mineral concentration in the suggested formulae in decreasing order is as follows Na> Fe> Zn> Mn> Ca> Cu> P> Mg>K> Se. The high levels of minerals in the suggested formulae may be due to the high concentration of minerals in their constituent plant parts [134,135,136,137,138]. In general, there is a general public interest in the availability of both macro and micro elements in the foods consumed daily. Such elements are necessary for normal physiological function, the deficiency of which causes serious metabolic abnormalities and the increase of which leads to toxicity [139]. Their presence of determined elements in suggested formulae show they are good source for essential minerals in particular the micro/trace elements (Fe, Zn, Cu, Mn, and Se) and were quite comparable with their recommended daily intake (RDI) Cu (2.2 mg/day), Fe (28–30 mg/day), Zn (15.5 mg/day) and Mn (5.5 mg/day) [140]. Such elements are biologically very important to the human through prevention and/or fighting several diseases including diabetes, anemia, immunodeficiency, cancer, atherosclerosis, heart diseases and aging [141,142]. Zn has a vital roles in different biological functions such as cell growth, division and maturation, cell membrane stabilization, and in DNA and RNA synthesis [143,144]. Cu participates in several important roles including the process of erythropoiesis, maturation, signal-mediated activity of immune cells, contributes to iron resorption in the digestive tract, catalyzes hemoglobin biosynthesis, and helping to incorporate heme iron [145]. Fe plays major roles in the synthesis of hemoglobin, the transport of oxygen from the lungs to the tissues of all organs of the body, and the synthesis of DNA and its function in the human immune system [146]. Se is a constituent of sveral selenoproteins, including thioredoxin reductases, glutathione peroxidases, and selenoprotein P. Selenoproteins play critical roles in thyroid hormone metabolism, DNA synthesis and reproduction. They also critically participate in the antioxidant defense systems inside and outside red blood cells, which fight free radicals (oxidative stress) that cause cell damage and exacerbate diseases of the brain and nervous system such as Parkinson's disease, Alzheimer's disease, and multiple sclerosis, in addition to cancer and heart disease [139,147]. Mn is found mostly in bones, the liver, kidneys, and pancreas. It has an important role in the metabolism of lipids and helps the body to form connective tissue, bones, blood clotting factors, and sex hormones [148]. On the other side, suggested formulae recorded high levels of macro elements including Ca, Mg and P subsequently may be a good source of minerals for bone formation. Also, the following can be noted in the present study Na/K ratio for formulae I and II are 105.68 and 115.10, respectively. According to the study of [149] when the ratio of Na/K is higher than one in diet, such diets have been linked with increased risk of hypertension and heart disease-related mortality. To address this problem, the Na/K ratio required for the suggested formulae must be supplemented with other K-rich nutrients. Also, formulae I and II analyzed for minerals have Ca/P ratio of 4.44 and 4.88, respectively. According to the study of [150] when the Ca/P ratio is higher than one in the diet, this diet is considered a good source of minerals needed for bone formation but with a ratio less than 0.5 it is considered a poor source.

Table 3. Minerals content of the suggested formulae (mg.100g^{-1} dry weight)

Element	Formula I		Formula II	
	Range	Mean ± SD	Range	Mean ±SD
K	24.67-28.99	26.07± 2.17 [a]	23.09-25.99	25.16±1.56 [a]
Na	2687.31-2819.2	2755.02±25.78 [b]	2659.54-2967.29	2895.78±19.26 [a]
Mg	30.19-37.89	34.89± 3.21 [a]	36.87- 46.98	39.99±5.14 [a]
Zn	790,67-810.78	793.71±10.12 [a]	708.56-720.90	712.11±10.67 [b]
Fe	923.78-975.77	953.19±20.76 [a]	976.78-998.56	982.77±8.65 [a]
Cu	143.76-158.10	153.71±7.34 [b]	168.67-179.78	170.34±5.51 [a]
Mn	412.67-432.89	425.95±9.24 [a]	440.10-467.20	446.35±11.67 [a]
Ca	140.01-147.84	142.88±4.09 [b]	197.56-210.65	202.04±6.60 [a]
Se	0.81-0.93	0.85±0.09 [b]	0.87 - 098	0.92±0.10 [b]
P	29.98-33.17	32.20±1.32 [b]	37.65-44.10	41.37±3.17 [a]

Each value represents the mean of four replicates ±SD. Means in the same raw with different superscript letters were significantly different at $p \leq 0.05$.

Table 4. Vitamins content of suggested formulae (mg.100g^{-1} dry weight)

Element	Formula I			Formula II			DRI
	Range	Mean ± SD	% of DRI	Range	Mean ±SD	% of DRI	
Vitamin A (β-carotene, μg/100g)	508.56 – 559.67	539.56 ± 16.21^{a}	59.95 (77.08)	440.67-461.04	451.74 ± 9.67^{b}	50.19 (64.53)	900 μg (700)
Vitamin C (Ascorbic acid, mg/100g)	20.98 – 26.19	25.78 ± 2.06^{a}	28.64 (34.37)	20.07 – 23.08	21.87 ± 1.08^{b}	24.3 (29.16)	90 mg (75)
Vitamin E (Tocopherols, mg /100g)	6.11 - 7.28	6.73 ± 0.68^{a}	44.87 (44.87)	4.81 – 5.80	5.17 ± 0.71^{b}	34.47 (34.47)	15 mg (15)
Vitamin B2 (Riboflavin, mg/100g)	1.92 – 2.18	2.01 ± 0.11^{a}	154.62 (182.73)	1.40 – 1.69	1.49 ± 0.14^{a}	114.62 (135.46)	1.3 mg (1.1)
Vitamin B6 (Pyridoxine, mg/100g)	1.06 – 1.28	1.14 ±0.09^{a}	67.06 (76.00)	1.20 – 1.49	1.27 ± 0.14^{a}	74.71 (84.67)	1.7 mg (1.5)
Vitamin B9 (Folate, μg/100g)	334.78 – 352.98	341.78 ± 7.45^{a}	85.45 (85.45)	255.65 – 284.50	268.65±14. 98^{b}	67.16 (67.16)	400 μg (400)

Each value represents the mean of three replicates ±SD. Means in the same raw with different superscript letters were significantly different at $p \leq 0.05$. DRI, Dietary Reference Intake; DRI for male, value without parenthesis; DRI for female, value in parenthesis.

3.3. Vitamins Content of Suggested Formulae

The vitamins concentration of suggested formulae is given in Table 4. Vitamin B2 was the most abundant vitamins in such formulae, followed by vitamins B9, B6, A, E and C. Vitamins including A, B2, B6 and B9 found in high concentration in formulae and were good comparable with their Dietary Reference Intake (DRI) in male by the rate of 59.95, 154.62, 67.06 and 85.45%, and 50.19, 114.62, 74.71 and 67.16% for formula I and II, respectively. These values were increased with their Dietary Reference Intake (DRI) in female to 77.08, 182.73, 76.00 and 85.45%; and 64.53, 135.46, 84.67 annd 67.16% for formula I and II, respectively. While other vitamins such C and E were found in moderate concentration and were partially comparable with their DRI. The high levels of vitamins in the suggested formulae may be due to the high concentration of vitamins in their constituent plant parts [66] [134,135] [151,152]. In nutritional point of view, vitamins are essential for life because we need them for good health and for growth. Vitamin A plays vital roles through protects the eyes from night blindness and age-related decline, may lower the risk of certain cancers, supports a healthy immune system, promotes healthy growth and reproduction, and supports bone health [153]. Vitamin C is essential for the development and maintenance of connective tissues, and plays an important role in bone formation and immune function, and and improves the absorption of nonheme iron i.e. the form of iron present in plant-based foods [154]. Vitamin E is important for reproductive health and central nervous system working properly. Also, it acts as an antioxidant during lipid peroxidation i.e. reacts with the peroxyl radical before it can attack the PUFA but generates a tocopheroxyl radical that must be reduced by other antioxidants, such as vitamin C. On the ther side, suggested formulae are good source of almost member of vitamin B including B2, b6 and B9 (folate). Vitamin B9 is involve in the metabolism of several amino acids, including, serine, methionine, glycine and histidine. The roles of folate and vitamin B12 in converting homocysteine to methionine, along with the role of vitamin B6 (pyridoxine) in converting homocysteine to cystathionine, continue to receive significant attention due to the low intake of these three vitamins (B6, B9, B12) especially vitamin B9 (folate), is inversely related to plasma homocysteine concentrations, and elevated plasma homocysteine concentrations (>15 μM) are associated with several serious diseases including premature coronary artery disease, premature occlusive vascular disease and cerebral or peripheral vascular disease [155,156] [157,158]. Another condition being investigated as possibly linked to poor folate status is dementia, including Alzheimer's dementia [159]. Folate deficiency is also suspected in the development (initiation) of some cancers, especially colon and colorectal cancers [160,161].

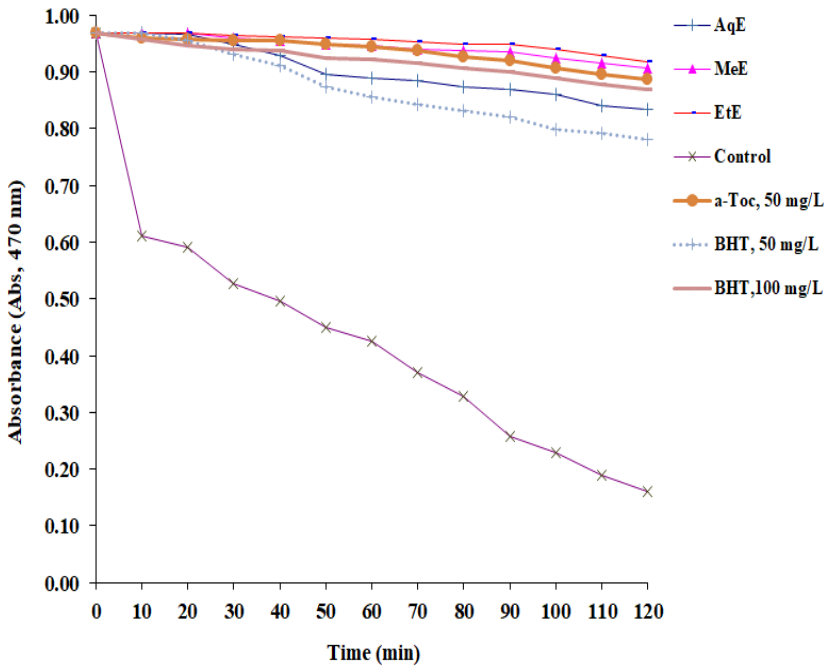
3.4. Bioactive Compounds and Antinutrients Content of Suggested Formulae

Bioactive/ antinutrient compounds contents in suggested formulae powder were shown in Table 5. Such data indicated that phenolics were the largest compound followed by polysaccharides, terpenoids, flavonoids, saponin, triterpenoids, oxalate, kaempherol and lycopene. Also, the content of these compounds except terpenoids, triterpenoids, and oxalate are higher in formula I than formula II. The high levels of bioactive compounds in the suggested formulae may be due to the high concentration of these compounds in their constituent plant parts. For example, onion skin and potato peel contain a wide spectrum of polyphenolic components and flavonoids including quercetin glycosides [17,23] [25,28] [35,135] [162,163]. Marjoram contains essential oil, phenolics and volatile compounds, [44,45,46]. Several bioactive compounds including trans-anethole, estragole, fenchone and polyphenolics were isolated from fennel [48]. Phenolic constituents and volatile compounds were determined in black seed [49]. Cinnamon contains condensed tanins, oil, coumarins, cinnamaldehyde and flavonoids [52,56]. Milk thistle fruits/seeds contain a mixture of flavonolignans collectively known as silymarin i.e. complex mixture of polyphenolic molecules, including seven closely related flavonolignans (silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, silydianin) and one flavonoid (taxifolin), and kaempferol, flavonoid phytoestrogen [66,85] [164,165]. Turmeric contains a wide variety of bioactive compounds including curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmeronols [69,74]. Molokhia contains phenolics, tannins, saponins and carotenoids [8,14] [22,85], Apricot kernels contains alkaloids and phenolic compounds [95]. Gum arabic contains polysaccharide composed of galactose, arabinose, rhamnose, glucuronic acid and 4-O-methylglucuronic acid, phenolics and dietary fiber [15,99] [103,105] Tomato pomace is rich in lycopene i.e. the principal carotenoid causing the characteristic red hue of tomatoes [34] [77,78]. In general, phenolics and flavonoids are playing several important biological effets including antioxidant and scavenging activities and inhibiting the low density lipoprotein oxidation [20] [134] [166,167,168]. Polysaccharides exhibited different biological activities including anticoagulant, anti-inflammatory, anti-obesity, anti-osteoporosis, antioxidant, hypocholesterolemic, hypolipidemic and antimicrobial activities [65] [168] [169,170] [171,172]. Lycopene is associated with antioxidant status, gap-junction formation, inhibition of cholesterol synthesis and prevent cancer and heart diseases [79,80]. Saponins, triterpene glycosides possess chemopreventive roles such lowers blood cholesterol, inhibits the growth of cancerous cells and enhances immune system [173,174]. Triterpenoids such as ursolic acid can serve as a starting material for synthesis of more potent bioactive derivatives, such as experimental antitumor agents [175]. Also, several studies indicated that polysaccharides and triterpenoids exhibit protective activities against liver injuries induced xenobiotics [176,177]. Finally, Kaempferol reduces the risk of chronic diseases, especially cancer, augments human body's antioxidant defense against free radicals, and modulates apoptosis, angiogenesis, inflammation and metastasis [178].

Table 5. Bioactive compounds and antinutrients content of suggested formulae

Component	Formula I		Formula II	
	Range	Mean ± SD	Range	Mean ± SD
Phenolics (mg gallic acid.100 g^{-1})	1114.54-1035.87	1123.67±11.78 ^{a}	971.56-1007.56	990.67±13.87 ^{b}
Flavonoids (mg catechin.100 g^{-1})	413.87-430.87	423.01±8.13 ^{a}	310.09-332.660	321.01±9.54 ^{b}
Lycopene (µg.100 g^{-1})	380.65-393.73	387.04±6.15 ^{a}	200.11-10.67	204.04±5.65 ^{b}
Polysaccharides (mg starch.100 g^{-1})	1089.56-1139.10	1109.59±19.56 ^{a}	903.67-928.56	917.59±11.54 ^{b}
Terpenoids (mg linalol.100 g^{-1})	485.87-520.76	504.19±13.90 ^{b}	620.90-6554.20	641.19±16.03 ^{a}
Triterpenoids (mg ursolic acid.100 g^{-1})	311.84-330.86	322.90±9.32 ^{b}	381.90-392.10	387.90±5.19 ^{a}
Saponin (mg.100 g^{-1})	352.81-369.02	361.50±7.79 ^{a}	197.89-208.02	203.50±5.90 ^{b}
Oxalate (mg.100 g-1)	90.65-306.90	297.12±8.45 ^{b}	357.5-370.11	364.12±7.39 ^{a}
Kaempherol (mg. 100 g^{-1})	22.01-25.62	23.70±1.56 ^{a}	9.10-12.02	10.89±1.09 ^{b}

Each value represents the mean of three replicates ±SD. Means in the same raw with different superscript letters were significantly different at $p \leq 0.05$



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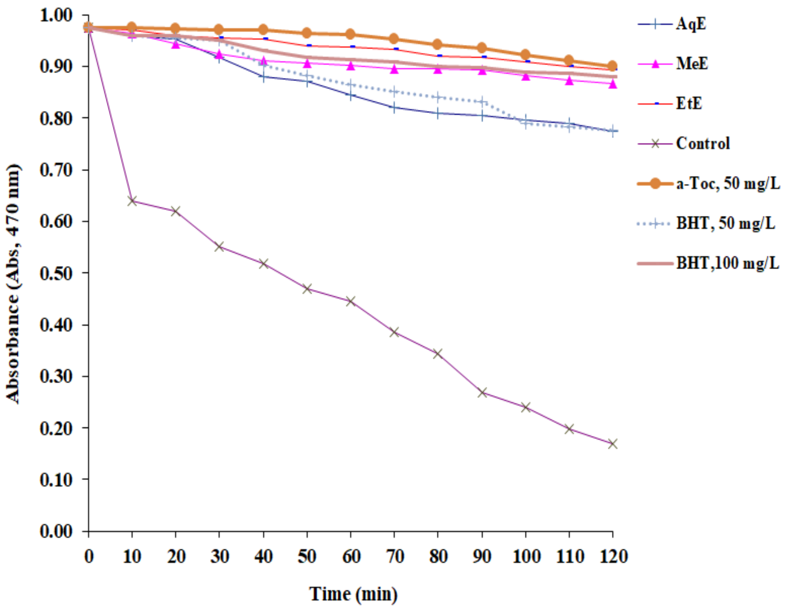

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Figure 1. Antioxidant activity of suggested formulae extracts and references/standard antioxidants assayed by the β-carotene bleaching (BCB) method. BHT, Butylated hydroxytoluene, EtE, Ethanol extract, MeE, Methanol extract, AqE, aqueous extract

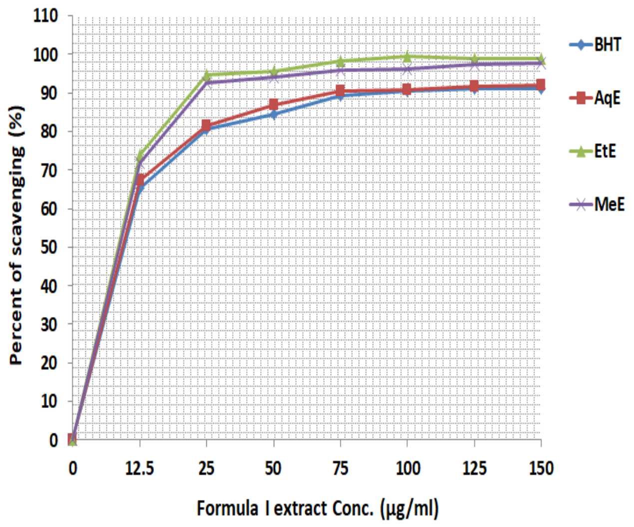
3.5. Biological Activities of Suggested Formulae Extracts

3.5.1. Antioxidant Activity

The antioxidant activity of suggested formulae extracts was assayed by β-carotene bleaching (BCB). This assay is based on the coupled oxidation of β-carotene and linoleic acid which estimates the relative ability of antioxidants to scavenge the radical of linoleic acid peroxide (LOO) that oxidizes β-carotene (lost the double bonds) in the emulsion phase. The decrease in absorbance of β-carotene in the presence of different suggested formulae extracts and references/standards antioxidants with the oxidation of β-carotene and linoleic acid is shown in Figure 1. The suggested formulae extracts showed considerable signifficat differences in antioxidant activity (AA). The trend of antioxidant activity score of formula I extracts in descending order is as follows: EtE> MeE> α-toc> BHT(100 mg/ml)>AqE> BHT(50 mg/ml). For formula II extracts as follow: α-toc> EtE> BHT(100 mg/ml)>MeE> BHT(50 mg/ml) >AqE. Thus, the extracts of formula I showed higher antioxidant activity than the formula II which exceeded the standard materials (α-toc and BHT) in its activity. Perhaps this difference in the degree of antioxidant activity between the suggested formulae may be due to the difference in the components included in the composition of each formula in terms of their type and concentration. Our previous studies with the others proved that the BCB method has been also used successively to evaluate the antioxidant activity in various plant parts including used in the suggested formulae preparation [9,10] [11,12] [18,19] [20,21,22] [66] [179,180]. All of these studies reported that the content of polyphenols, flavonoids, carotenoids, tannins, saponins, lycopene, kaempferol and polysaccharides, such as those found in a highly content in extracts of the proposed formulae, are highly correlated with the antioxidant activities. Finally, results of the present study propose that all the extracts from suggested formulae showed antioxidant activity through radicals scavenging.

3.5.2. DPPH Radical Scavenging Activity

The free radical scavenging activity of the suggested formulae and butalyted hydroxyl toluene (BHT) as a standard are illustrated in Figure 2 and Table 6. From such data it could be noticed that ethanol extracts possessed the highest activity followed by methanol and aquatic ones. At a concentration of 100 μg/mL, the radicals scavenging activity of aquatic, ethanol and methanol extracts for formula I were 90.65, 99.56 and 96.07%, respectively, and for formula II were 88.83, 94.92 and 93.93%, respectively, whereas, BHT standard was 90.53%. For the IC50, the aquatic, ethanol and methanol extracts for formula I were recorded 9.26, 8.46 and 8.68 μg/mL, respectively and for formula II were 9.94, 8.71 and 8.97 μg/mL, respectively, while, BHT standard was.9.60 μg/mL. Therefore, the free radical scavenging activity of formulae extracts and standard was in the following order: ethanol extract > methanol extract > aquatic extract > standard (BHT) for formula I and ethanol extract > methanol extract > standard (BHT) > aquatic extract for formula II. The theory of the DPPH radicals scavenging activity test is based on measurement of the a absorption of diene conjugation in the presence of DPPH [181]. Numerous previous studies revealed that DPPH assay has been used successfully to evaluate the scavenging activity of several plant parts including used in the suggested formulae preparation [20] [34] [133,168] [171,182] [183,184,185]. Thus, data of the present study propose that all the suggested formulae extracts showed free radical scavenging activity which due to their rich in different categories of bioactive compounds knowing as antioxidants including phenolics, polysaccharides, carotenoids, Kaempherol, lycopene, tannins, flavonoids etc. Such data are in accordance with that observed by [132,133] [186]. Finally, present study data suppose that all suggested formulae extracts showed radical scavenging activity through electron transfer or hydrogen donating ability.



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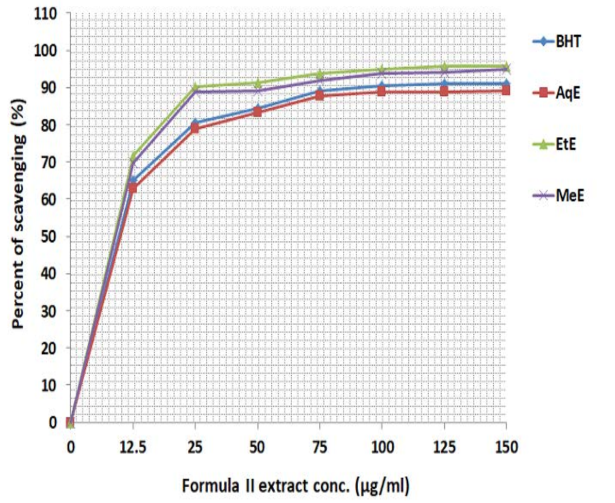

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* Each value represents the mean value of three replicates. BHT, Butylated hydroxytoluene, EtE, Ethanol extract, MeE, Methanol extract, AqE, aqueous extract

Figure 2. DPPH radical scavenging activity (%) of suggested formulae extracts and standard (BHT)*

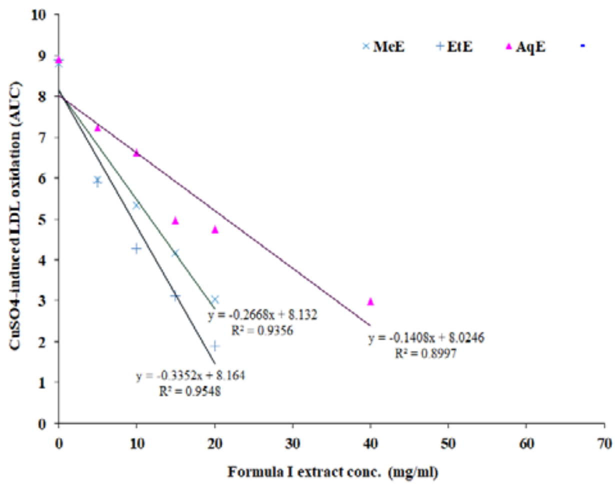
Table 6. IC_{50} (DPPH) of suggested formulae extracts and BHT (Standard)

Name of sample	BHT	AqE	EtE	MeE
IC_{50} (µg/mL) of Formula I	9.60^{a}	9.26^{a}	8.46^{b}	8.68^{ab}
IC_{50} (µg/mL) of Formula II	9.60^{a}	9.94^{a}	8.71^{b}	8.97^{ab}

* Each value represents the mean value of three replicates ±SD. Values with different superscript letters in the same raw are significantly did different at p≤0.05. BHT, Butylated hydroxytoluene, EtE, Ethanol extract, MeE, Methanol extract, AqE, aqueous extract

3.5.3. Inhibition of Low Density Lipoprotein (LDL) Oxidation

Data illustrated in Figure 3 shown the dose-dependent inhibition of $CuSO_4$-induced LDL oxidation *in vitro* by suggested formulae extracts. Such data indicated that the inhibitive action of suggested formulae extracts against $CuSO_4$-induced LDL oxidation, as evidenced by decreased conjugated dienes production in a dose-dependent behavior. The comparative study amongst suggested formulae extracts showed that the aquatic, ethanol and methanol extracts acted more dramatically in protecting LDL against oxidation. The protecting LDL against oxidation activity of formulae extracts was in the following order: ethanol extract > methanol extract > aquatic extract. Such data with the others indicated that such protecting effect could be attributed to the various bioactive constituents as antioxidants/scavengers (phenolics, polysaccharides, carotenoids, Kaempherol, lycopene, tannins, flavonoids etc.) contained in such suggested formulae extracts [34] [132,133] [168,171,186]. In general, ROS cause cellular damage membranes (cell wall, mitochondria, lysosomes etc.) by peroxiding lipid moieties, particularly the polyunsaturated fatty acids in a chain reaction known as lipid peroxidation [187]. Thus, the inhibition of lipid peroxidation is considered the most important parameter of antioxidant activity. Data of the current study show lipid peroxidation inhibition activity of all suggested formulae extracts. These results indicated that suggested formulae extracts can prevent cellular damage caused by free radicals through slump the chain reactions responsible for lipid peroxidation. With the same context, several authors noticed that phenolic compounds such as detected in formulae extracts exhibited a protecting LDL against oxidation through increasing the levels of reduced glutathione (GSH) and inhibition of NADPH-dependent lipid peroxidation in liver [171,184,188]. Also, several studies reported that the oxidative modification of lipoproteins hypothesis postulates that LDL oxidation plays a key role in early atherosclerosis [189,190]. The oxidized LDL is atherogenic due to its cytotoxic roles toward arterial cells and stimulates the monocytes to be adhesive to the endothelium which leads to the development of atheromatous plaques [191] Our present data proved that the formulae extracts could be used successfully as a promising agents in atherosclerosis prevention through inhibiting LDL oxidation process.



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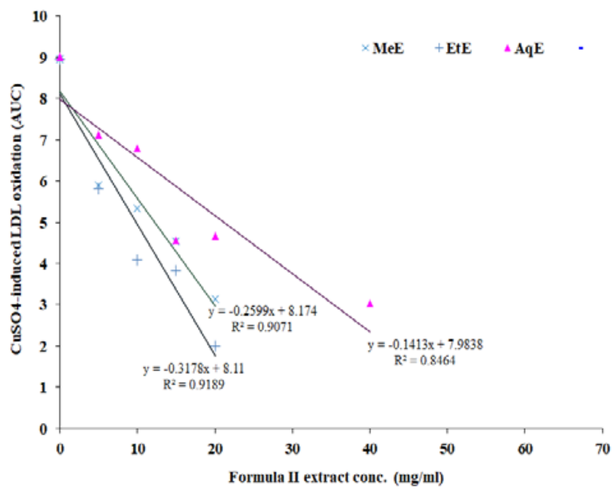

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Figure 3. Dose-dependent inhibition of CuSO4-induced LDL oxidation *in vitro* by of suggested formulae extracts. EtE, Ethanol extract, MeE, Methanol extract, AqE, Aquatic extract

3.6. Relationship Between Bioactive Compounds Content and Antioxidant Activities of Suggested Formulae Extracts

Table 7. The correlation coefficients (r^2) between bioactive compounds content and antioxidant activities of suggested formulae extracts

Bioactive compound	Antioxidant activity (β-carotene bleaching rate)	DPPH radical scavenging activity	Inhibition of low density lipoprotein (LDL) oxidation
Phenolics	0.9417 ±0.0132^{aA}	0.9389 ±0.0199^{aA}	0.9498 ±0.0156^{aA}
Flavonoids	0.8442 ±0.0073^{dB}	0.8902 ±0.0252^{bA}	0.8291 ±0.0191^{cB}
Lycopene	0.7917 ±0.0196^{eA}	0.8102 ±0.0173^{dA}	0.7942 ±0.0257^{dA}
Polysaccharides	0.9293 ±0.0152^{abA}	0.9003 ±0. 0212^{bB}	0.8547 ±0.0210^{bC}
Terpenoids	0.8304 ±0.0256^{dB}	0.8703 ±0. 0179^{bA}	0.8210 ±0.0165^{cB}
Triterpenoids	0.8701 ±0.0143^{cA}	0.8865 ±0.0198^{bA}	0.8023 ±0.0342^{cdB}
Saponin	0.9091 ±0.0131^{bA}	0.8942 ±0.0263^{bA}	0.8131 ±0.0231^{cB}
Oxalate	0.4131 ±0.0054^{gA}	0.2261 ±0.0163^{eB}	0.39017 ±0.0099^{eA}
Kaempherol	0.75154 ±0.0284^{fB}	0.8392 ±0.0252^{cA}	0.7907 ±0.0073^{dB}

Each value represents the means of three replicates ± SD. Means with different superscript small letters in the same columns are significantly different at p≤0.05. Means with different superscript capital letters in the same rows are significantly different at p≤0.05.

The correlation coefficients (r^2) between bioactive compounds content and biological activities [β-carotene bleaching rate, free radical scavenging efficiency and inhibition of LDL oxidation] of suggested formulae were illustrated in Table 7. Total phenolics, polysaccharides, saponin content of the extracts showed significant ($p\leq 0.05$) and strong positive correlation with the antioxidant activity [β-carotene bleaching assays]. The rest of bioactive compounds (Flavonoids, triterpenoids, terpenoids, lycopene kampherol and oxalate) content showed significant ($p\leq 0.05$) and moderate positive correlation for the same relationship. For scavenging activity assay, all bioactive compounds except oxalate exhibited significant ($p\leq 0.05$) and strong positive correlation. The results also showed that the effectiveness of inhibiting LDL oxidation depends to a large extent on both antioxidant and scavenging activities, in addition to other factors that were not addressed in this study. Data of the present study are according with that reported by [192] for DPPH radical scavenging, even though the authors performed a different extraction procedure (methanol :water, 80:20, v/v). Thus, it could be proposed that bioactive compounds determined in the suggesting formulae play a more prominent role in biological activates i.e. antioxidant and scavenging activities and lipid peroxidation inhibition.

3.7. Antibacterial and Antifungal Activities of Suggested Formulae Extracts

Table 8. Antibacterial and antifungal activities of suggested formulae extracts

Extract	Bacteria		Fungi
	Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
Formula I			
AqE	7.96±0.3*	15.17±0.8^{b}	13.56±0.9^{b}
MeE	10.13±0.4*	20.16±1.1^{a}	19.04±0.6^{a}
EtE	10.88±0.5*	21.88±1.7^{a}	19.63±1.2^{a}
Formula II			
AqE	8.76±0.3*	15.09±0.8^{b}	12.41±0.9^{b}
MeE	9.87±0.4*	18.45±1.1^{a}	17.80±0.6^{a}
EtE	11.03±0.5*	20.09±1.7^{a}	18.89±1.2^{a}

Each value represents the mean ±SD. Means with different superscript letters in the same raw are significantly different at p≤0.05. *Diameter of the inhibition zone less than 10 mm means absence of activity. EtE, Ethanol extract, MeE, Methanol extract, AqE, Aquatic extract

The antibacterial and antifungal activities of suggested formulae extracts were illustrated in Table 8. From such data it could be noticed that the highest activity (inhibition zones) against the Gram-negative bacteria *Escherichia coli* was recorded for ethanol extract (10.88- 11.03) with no activity for aquatic extract. For gram-positive bacteria *Staphylococcus aureus* the highest activity (20.09 – 21.88 mm) was recorded for ethanol extract followed by methanol extract (18.45- 20.16 mm) and aquatic extract (15.09-15.17 mm). The same behavior was observed for the antifungal activity against *Candida albicans.* Also, formula I is higher in all microorganisms inhibition than formula II. Such variations recorded in the amount of the antibacterial and antifungal activities measured (inhibition zones) for the suggested formulae extracts could be mainly due to the difference in polarities of extracting solvents. The ethanol and methanol solvent are higher in improving the recovery of the bioactive compounds in suggested formulae than water, subsequently increases their efficiency in inhibiting bacteria and fungi. Such data are relatively accordance with that reported by several authors have been working in various plant parts contained the same bioactive compounds found in the suggested formulae [20] [168]. With this context, Kamenarska et al., [193] reported that the toluene (non-polar compounds, including volatile compounds) and methanol:chloroform (1:1) extracts (compounds with average polarity) showed a moderate activity only against the gram-positive bacteria. Also, the ethanol extract (more polar compounds) showed not only a moderate activity against gram-positive bacteria but also a moderate antifungal activity. Finally, all the previous data confirmed the potential use of suggested formulae as good sources of antibacterial and antifungal agents.

4. Conclusion

Data of the current study supported our hypothesis that the suggested formula of plant parts contains several classes of bioactive compounds including phenolics, polysaccharides flavonoids, terpenoids, triterpenoids, lycopene, kaempherol, saponins, oxalate, etc., with other compounds that are responsible for their different biological activities. The biological activities studied enhanced here including antioxidant and scavenging activities, inhibition of LDL oxidation and antibacterial and antifungal activities. Such important biological activities could be played important roles in strategies to prevent/combat/treat several diseases, especially those for which oxidative stress is one of the mechanisms for its occurrence. Therefore, the present study recommended like of these suggested formulae powders and/or extracts to be included in our daily diets, drinks, food supplementation and pharmaceuticals.

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Conflict of Interests

Authors declared no competing of interest whatsoever.

Ethical Considerations

The ethical issues of the current work was reviewed and approved by the Scientific Research Ethics Committee, Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval # 23- SREC- 05-2022).

Authors' Contribution

Yousif Elhassaneen cooperated in proposing and improving the study protocol, following up on the laboratory experimental part, retrieving conceptual information, reviewing and validating the results and statistical analyses, preparing a draft of the manuscript, conducting a critical review to intellectually organize the content and granting approval to publish the final version of the manuscript. Eman Aram conducted the laboratory experiments, collected, tabulated, analyzed and interpreted the results. She was also involved in retrieving conceptual information and preparing the draft of the manuscript. Ghada ELBassouny cooperated in proposing the study protocol, retrieving conceptual information, validating the study results, and preparing the draft manuscript. Omar Emam cooperated in improving the study protocol, retrieving conceptual information, and submitting contributions to the concept and design of the work.

Abbreviations

AA: antioxidant activity, AAPH: 2,2′-Azobis(2-methylpropionamidine) dihydrochloride, Abs: absorbance, AqE: aqueous extract, BHT: butalyted hydroxyl toluene, BCB: β-carotene bleaching, CA: catechine, DMSO: dimethyle sulfoxide, DNA: deoxyribonucleic acid, DPPH: 2,2-diphenyl-1-picrylhydrazyl, DW: dry weight, EtE: ethanol extract, GA: gallic acid, GAE, gallic acid and equivalents, G.D.R.: grams consumed of food (wet weight basis) to cover the daily requirements of adult man (63 g) in protein and energy, GSH: reduced glutathione, LDL: low density lipoprotein, MtE: methanol extract, P.S: percent satisfaction of the daily requirement of adult man in protein and energy, RNA: ribose nucleic acid, SD: standard deviation, T.N.: total nitrogen.

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