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Evaluation of Antioxidant Efficiency of *Croton tiglium* L. Seeds Extracts after Incorporating Silver Nanoparticles

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THIS STUDY aimed to enhance efficiency of the different *Croton tiglium (C. tiglium)* seeds extracts by incorporation of silver nanoparticles (Ag-NPs) through raised up cytotoxicity against growth of human colon cancer cells. *C. tiglium* seeds contain various phytoconstituents e.g., carbohydrates (glycosides), flavonoids, sterols (triterpenes), alkaloids and proteins. The protein was hydrolyzed in the dried aqueous seeds extract into free amino acids, the essential amino acids (20.71%) & nonessential amino acids (79.29%). Furthermore, the mucilage in the dried aqueous seeds extract and the lipoidal contents in the saponifiable matter of the petroleum ether extract were represented by total identified sugars represent ~81.21 % of the total mucilage hydrolyzate. The lipoidal contents in the saponifiable matter of the petroleum ether extract were represented by total saturated fatty acids (13.68%), total monoenoic fatty acids (34.49%), total dienoic fatty acids (45.76%) and total identified fatty acids (93.93%).

Moreover, the unsaponifiable matter was represented by saturated hydrocarbons (40.73%), unsaturated hydrocarbons (18.93%), fatty alcohols (10.08%), sterols (10.61%) and total identified compounds (80.35%). In addition, there were four compounds (β sitosterol, α-amyrin, Oleanolic acid and 3-*O*-β-D-glucopyranosyl-β-sitosterol (daucosterol)) isolated from the petroleum ether extract. Additionally, incorporation of Ag-NPs into the extract caused no toxicity on the experimental animals when administrated orally. It was found that the median lethal dose (LD_{50}) of the ethanolic, petroleum ether and aqueous seeds extract-Ag nanocomposites was about 7.95, 5.2 and 65 ml/Kg, respectively.

Keywords*:* *Croton tiglium* L. Seeds, Silver nanoparticles, Polyphenols, Scavenging activity, Anticancer activity.

Introduction

Croton tiglium L. belongs to the family Euphorbiaceae that occur in tropical and temperate regions all over the world and includes about 280 genera and 8000 species which occur in tropical and temperate regions all over the world [1]. It is widely used in ethnomedicine for the treatment of several cancer diseases [2]. Seeds, leaves, bark and root of *C. tiglium* are used in traditional medicine for treatment of constipation, dyspepsia, dysenteriae, gastrointestinal disorders, intestinal inflammation, rheumatism, peptic ulcer, visceral pain, headache, purgative, colds, fever, worms, ascities and intracranial hemorrhage and in snake poisoning[3-5]. It was reported that *C. tiglium* seeds are well-known by its toxicity (severe purgative action). This because the seeds oil contains phorbol esters and crotonic acid along with the fatty acids in addition to presence of the active phytoconstituents. These constituents are oil soluble and it is necessary to remove of

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reduce level of these constituents to reduce the *C. tiglium* seeds toxicity during the purification process [6-8].

An attempt was made by High-performance liquid chromatography (HPLC) for identification and quantification of the toxic principles along with other physiochemical parameters and responsible for the severe purgative action of *C. tiglium* seeds [7]. Moreover, the ethanolic seed extract of *C. tiglium* exhibited strong antidermatophytic activities. It was found that crude extracts from these plant seeds were active against *Trichocomaceae* (*Aspergillusniger* and *A. tamarii*) and *Mucoraceae* (*Mucormucedo* and *Rhizopussolani*) [9]. Thereafter, the ethanolic seeds extract of *C. tiglium* exhibited atopical application on treating skin fungal infection and formulation of the extracts into shampoo or soap [10].

It was postulated that *C. tiglium* seeds extract exhibited high antioxidant activity due to the presence of some other phytochemicals such as ascorbic acid, tocopherol and other pigments [11]. 12-O-tetradecanoylphorbol-13-acetate is major active constituent in the croton oil which was isolated from seeds of this plant. It is used for treatment of the solid tumors due to its ability to inhibit the growth, stimulate apoptosis in prostate, breast, colon and lung cancer diseases [12]. In 2014, Yumnamcha *et al*. [13] emphasized that aqueous *C. tiglium* seeds extract cause increase plasmid DNA strand breakage in a dose dependent manner. They found that it is necessary for the plant extract need to be evaluated before it could be used for therapeutic purposes. The recent study showed that *C. tiglium* seeds extracts have no significant alterations in the biochemical measurements but they have little effect on some haematological indices [14].

Development of polymer-metal nanocomposites containing metal nanoparticles (MNPs) is considered to be one of the most promising solutions to their inherent stability problem. Incorporation of MNPs into polymeric matrices showed valuable properties in many practical applications [15-19]. Synthesis of silver nanoparticles (Ag-NPs) by reduction of aqueous silver nitrate into Ag-NPs during exposure to plant extracts can be easily monitored by using UV-visible spectrophotometer [20]. The plant extracts with Ag-NPs exhibited good antioxidant activity at lower concentrations [21]. Recently, it was found that incorporation of Ag-NPs in the plant extract increased the total phenolic compounds and total flavonoids. It was found that plant Ag-NPs showed a higher antioxidant and antimicrobial activity compared to plant extract alone or silver nitrate [22].

The present study aimed to evaluate the phytochemical and biological efficiency of different *C. tiglium* seeds extracts. Consequently, efficiency of the different extracts was studied after incorporation of the nanoparticles.

Materials and Methods

Preparation of the plant materials

The dried *C. tiglium* seeds were obtained from Agricultural Research Center, Giza, Egypt and dried in an incubator at 50 °C for 72 hrs. The dried seeds were crushed into powder in an electric blender.

Pharmacopoeial constituents and vitamins (fat and water soluble)

Moisture content was determined by heating finely powdered seeds of *C. tiglium* in the oven at 105°C for 3hrs. Furthermore, the ashes contents (total, water soluble and acid-insoluble ashes) were assayed in the powdered seeds by method suggested by Kirk and Sawyer [23]. Fat and water soluble vitamins were analyzed according to method suggested by Hasan *et al*. [24] using High Performance Liquid Chromatographic system (Shimadzu-UFLC Prominence), equipped with an auto sampler (Model-SIL 20AC HT) and UV-Visible detector (Model-SPD 20A).

Preliminary phytochemical screening tests

Wide range of the common phytoconstituents was determined in the plant seeds according to recommended and referenced methods. These constituents were represented by carbohydrates and /or glycosides [25], free and combined flavonoids [26], coumarins, saponins, alkaloids, nitrogenous compounds, sterols and /or triterpenes [27], tannins [28], proteins [29] and anthraquinones [30].

Preparation of different C. tiglium seeds extracts for phytochemical studies

Preparation of alcoholic extract

The powdered seeds had been extracted by cold maceration in successive portions of 80% ethyl alcohol at room temperature (cold maceration). The extraction mixture was filtered through a *Whatman No. 1* filter paper and then concentrated to dryness in a rotary evaporator at 45°C under reduced pressure.

Preparation of petroleum ether extract

The dried seeds powder had been separately extracted with petroleum ether (60-80°C). The extract was separately filtered through a *Whatman No*. 1 filter paper, and then concentrated to dryness in a rotary evaporator at 45°C under reduced pressure.

Preparation of aqueous extract

The dried powdered seeds have been extracted by distilled water. The extract was separately filtered through a *Whatman No*. 1 filter paper, and then concentrated to dryness in a rotary evaporator at 50°C under reduced pressure.

Chromatographic analysis of total phenolics

The different phenolic compounds were identified by high pressure liquid chromatograpahy (HPLC) (Shimadzu-UFLC Prominence). This technique is consisting of two units. One is responsible for separation of the compounds according to the difference in polarity and the other is responsible for detection of these compounds. It is equipped with an auto sampler (Model-SIL 20AC HT) and UV-Visible detector (Model-SPD 20A) (Japan). The separation process was carried out through analytical column of an Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase is consisting of solvent system of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). Before the chromatographic run, all samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI). Fifty micro litre (50 µl) from each extract (alcoholic and water aqueous) was injected automatically by the injector piece. The flow rate was kept at 0.8 $m.min^{-1}$ for a total run time of 70 min and the gradient program was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively. When the chromatographic run was finished, the peaks were identified by congruent retention times and UV spectra and compared with those of the standards.

In the dried aqueous extract, total carbohydrate content was quantified as glucose by phenol sulphuric acid method [31]. Consequently, the mucilagehas been separately isolated [32] then identified separately through specific testes [33, 34]. Furthermore, the Mucilage was hydrolyzed and purified according method of Chrums and Stephen [35]. The polysaccharides were analyzed in the hydrolysate using Gas Liquid Chromatography (GLC) (model HP 6890) equipped with flame ionization detector at 270°C. The analysis was carried out using ZB-1701 column (30m×0.25m×0.25µm), 14% cyanopropyl phenyl methyl, the carrier gas is helium at flow rate of 1.2 ml/min under pressure 10.6 psi and velocity of 41 cm/sec. The injector chamber temperature was -250°C. Quantitative determination was based on peak area measurement while qualitative identification was carried out by comparison of the retention times of the peaks with those of the authentic sugars.

In addition, the total protein content has been estimated by determining the nitrogen content using micro-kjeldahl method suggested by Pearson [36] using Markham distillation apparatus. The proteins were isolated, for isolation and purification of the polypeptides according to method described by El-Gengaihi *et al*. [37]. The dried crude polypeptides have been separately dialyzed by membrane (dialysis bag) and the non-dialyzable fraction was collected and dried. Consequently, the polypeptides were hydrolyzed into amino acids that have been separated by HPLC then analyzed by amino acid analyzer (model Eppendorf-Germany LC 3000) according to method suggested by Widner and Eggum [38].

In the petroleum ether extract, the total steroidal and terpenoidal contents were estimated quantitatively by the spectrophotometric method which is based on measuring intensity of the color developed when sterols and triterpenes react with Lieberman-Burchard reagent. Percentages of steroidal and terpenoidal have been calculated as β-sitosterol and β-amyrin, respectively with reference to a pre-established standard calibration curve [39]. The extract residues were saponified and concentrated according to method suggested by Tsuda et al. [40].

Moreover, the total free fatty acid content was liberated from the unsaponifiable matter then quantified. The fatty acids in the residue were converted into the methyl ester form based on method suggested by Finar [41]. Consequently, the fatty acid methyl esters and unsaponifiable matter were subjected to gas chromatograph coupled with a mass spectrometer (model Shimadzu GC/MS–QP5050A). The constituents have been identified by comparison of their spectral fragmentation patterns with those of the available database libraries Wiley (Wiley Int.) USA and NIST (Nat. Inst. St. Technol., USA) and/

or published data [42]. Quantitative determination was carried out based on peak area integration.

The dried petroleum ether extract was submitted to top of a column packed with 175 g activated silica (120 cm height x 2.5 cm i.d.). Elution was successively carried out by different ratios of petroleum ether (60-80°C) and chloroform with increasing polarity. The fractions were successively collected and concentrated then screened by thin layer chromatography (TLC) using benzene: ethyl acetate (8:2) as solvent system. The TLC plates were examined under UV-254 nm and visualized. The similar fractions were combined then evaporated under reduced pressure at a temperature not exceeding 40°C. Thereafter, the isolated compounds were subjected to physical, chemical, chromatographic and spectral analyses (UV, MS, IR, ^{1}H and ^{13}C NMR) as well as comparison with the available reference standards and available published data.

Preparation of C. tiglium seeds extracts silver nanocomposites

The Ag-NPs were prepared through reducing silver nitrate ($AgNO_3$) with ethylene glycol (EG) in the existence of polyol. In a classic method, 10 mL of EG were refluxed at 160 ºC for 25 min. Then, 5 mL solution of $AgNO_3$ in EG and 10 mL solution of 0.15 M PVP in EG containing 0.03 mM $MnCl_2$ were concurrently injected into the flask over a period of 10 min, the reaction mixture was further refluxed and vigorously stirred at 160 ºC for 60 min. The reaction mixture was then cooled to room temperature. The product was centrifuged at 3000 rpm for 5 min, and then washed with acetone and ethanol for three times. The Ag-NPs were collected and dried in oven at 70 ºC then the Ag-NPs were added to the *C. tiglium* seeds extracts by different concentrations to form *C. tiglium* seeds extracts silver nanocomposites.

In vitro antioxidant and cytotoxic activities of the different extracts

Total polyphenolic compounds

The total polyphenolic compounds were estimated in the different *C. tiglium* seeds extracts before and after incorporation of silver nanoparticles according to Singleton and Rossi [43] by using folin ciocalteu reagent purchased from Sigma Chemicals Co. Concentration of the total polyphenols was calculated as a gallic acid equivalent from the calibration curve of gallic acid standard solutions obtained from Sigma Chemicals Co. covering the concentration range between 0.2 and 1.0 mg / ml.

Total antioxidant capacity

Total antioxidant capacity of extract was evaluated through the assay of the green phosphate/ Mo^{5+} complex according to the method described by Prieto *et al*. [44]. Antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight.

Total reducing power

The total reducing power was determined according to method suggested by Oyaizu [45]. The absorbance was measured at 700nm against blank prepared without adding extract. Ascorbic acid at various concentrations was used as standard. A high absorbance of the reaction mixture at 700nm indicates a higher reducing power.

Free radical scavenging activity

DPPH radical-scavenging activity

Percentage of the antioxidant activity was evaluated by method described by Brand-Williams *et al*. [46] using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) for initiation of the free radicals and absorbance of the resulting solution was measured spectrophotometrically at 517 nm.

ABTS radical scavenging assay

For 2, 2'-azinobis-(3- ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, the procedure followed the method suggested by Arnao *et al*. [47] with some modifications. The extracts were allowed to react with ABTS solution and the absorbance was taken at 734 nm after 7 min using a spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of ascorbic acid.

Anticancer activity

Cytotoxic activity test (*In vitro* bioassay on human tumor cell lines) was conducted and determined. It was performed on human colon carcinoma cell line according to protocol suggested by Vichai and Kirtikara [48].

Characterization of the prepared Ag-NPs

X-ray diffraction (XRD)

The crystal structure of the filler powders was determined using a Philips X-ray diffractometer (PW 1930 generator, PW 1820 goniometer) equipped with Cu Kα radiation (45 kV, 40 mA, with $\lambda = 0.15418$ nm). The scans of the analysis were run in 2θ range of 5 to 80º with step size of 0.02 and step time of 1s.

Transmission Electron Microscope (TEM)

The morphological and particles size of

prepared samples were demonstrated by using TEM model JEM-1230, Japan, operated at 120 kV, with maximum magnification of $600X10^3$ and a resolution until 0.2 nm. A drop of an aqueous dispersion of the prepared samples was placed on a carbon-coated copper grid and allowed to dry in air before characterization.

UV-spectroscopy of the prepared Ag-NPs

UV-spectroscopy was carried by Shimadzu UV-Vis recording spectrophotometer UV-240.

DLS measurements

Particle size distribution was measured using Particle Sizing Systems, Inc. Santa Barbara, Calif., USA.

Experimental

Ethical Statement

The experimental design and animal handling were performed according to the experimental protocol which was approved by Institutional Animal Ethics Committee of National Research Centre, Dokki, Giza, Egypt and were conducted in accordance with guidelines as per "Guide for the care and use of laboratory animal" and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals.

Median lethal dose of different extracts (LD50)

The different *C. tiglium* seeds extracts (after incorporation of Ag-NPs) were evaluated separately after calculating the LD_{50}. Two hundred and forty adult albino mice (weight 20-25 g) were used to study acute toxicity. Animals were divided into 10 groups (8 mice in each group) for each extract. The groups were treated orally by stomach tube with rising doses of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 ml/mice of extract-Ag nanocomposites. Mortality was recorded after 24 hrs of extract treatment. The LD_{50} was calculated for each extract-Ag nanocomposites using equation suggested by Paget and Barnes [49].

Result and Discussion

Evaluation of phytochemical and physico-chemical properties of C. tiglium seeds

The phytochemical and physico-chemical evaluation of *C. tiglium* seeds provide useful information to verify the pharmacognostical identity of seeds of this plant and to carry phytochemical and physicochemical investigations of these seeds [50].

Table 1 revealed data of the physiochemical parameters (moisture, total ash content, water-soluble ash and acid insoluble ash) which determined in the dried *C. tiglium* seeds powders following procedure of the Egyptian pharmacopoeia. These constants could be used as significant criteria for the identity and purity of these plant seeds. Elevation of the ash contents during the purification process might be attributable to loss of oil soluble constituents in the plant seeds [51].

Working with crude extracts, means working with complex mixtures of biologically active compounds, some of the compounds in such a mixture can be genotoxic or antigenotoxic [13]. For this reason, it is necessary to undergo phytochemical screening to predict the potential health hazards occurred as a result of using the plant for medicinal purposes. Phytochemical constituents are non-nutrient, bioactive, secondary metabolites, naturally occurring plant compounds present in *C. tiglium* seeds and are considerably diverse [52].

Phytochemical screening of C. tiglium seeds

These constituents are commonly used for medicinal purposes as analgesic, antimalarial, bactericidal and antiseptic [53]. In the present study, the air-dried *C. tiglium* seeds powder subjected to the phytochemical screening tests for the detection of various bioactive compounds using chemical methods. As illustrated in **Table 2**, the air-dried seeds powders contain various phytoconstituents such as carbohydrates (glycosides), flavonoids, sterols (triterpenes), alkaloids (nitrogenous compounds) and proteins. This was in accordance with the study documented by Lopes *et al*. [54]. It was speculated that certain saponin mixture and alkaloids might be responsible for the genotoxic effects of *C. tiglium* seeds.

It was reported that certain group of bioactive compound like alkaloids and saponin mixtures are responsible for the DNA damage [55]. Further studies with more advanced practical techniques are required for confirming the genotoxic potential of *C. tiglium* seeds. Moreover, Salatino et al. [52] reported that terpenoids are the predominant secondary metabolite constituents in seeds of this plant. Triterpenoids, either pentacyclic or steroidal, have frequently been reported for seeds of *Croton* species. From medicinal point of view, all the seeds extracts revealed the presence of alkaloids, flavonoids, terpenoids, cardiac glycosides, tannins, glycoside and saponins which are considered very important components [56].

TABLE 1. Percentages of certain pharmacopoeial constants of *C. tiglium* seeds.

Pharmacopoeial constants (%)			
Moisture	**Total Ash**	**Water soluble ash**	**Acid insoluble ash**
7.5	15	8.6	5.7

TABLE 2. Phytoconstituents of *C. tiglium* seeds.

Constituent	Result
Carbohydrates (Glycosides)	++
Flavonoids	+
Saponins	-
Tannins	-
Sterols (Triterpenes)	++
Alkaloids (Nitrogenous compounds)	+
Proteins	+
Anthraquinones	-

(-): Absent, (+): Present, (++): Appreciably present.

Presence of carbohydrate in the seeds extract could be a good source of energy [57]. Also, presence of tannins and alkaloids in *C. tiglium* seeds with high quantity may be responsible for antiemetic activity. Saponins are responsible for antibiotic activity in addition to its potential efficiency to inhibit growth of cancer cells [58, 59]. Flavonoids play a vital role against inflammation, platelet aggregation, allergies and microbial infection [60].

Study the physical and chemical characters of the C. tiglium seeds

As presented in **Table 3**, it was found that the highest yield was obtained in petroleum ether extract which appeared oily yellowish brown extract before incorporation of Ag-NPs, this because the *C. tiglium* seeds could be a good source of fixed oil and essential fatty acids [57]. Also, petroleum ether extract contains the highest amount of sterol. This finding was supported by the study carried out by Lan et al. [61] who reported that the oil obtained by extracting of the *C. tiglium* seeds with petroleum ether was subjected to methyl-esterification or dilution with ethyl ether. They postulated that the linoleic acid, oleic acid and eicosenoic acid were the main components which were identified in croton oil as a result of GC/MS analysis of in methyl-esterified samples in addition to the aromatic compounds (phorbol esters). Ganer *et al*. [50] emphasized that toxicity of *C. tiglium* seeds might refer to presence of oil soluble phorbol esters and crotonic acid in addition to the other constituents. During the purification process, the toxicity reduced through removal of these constituents. Thereafter, seeds of this plant became suitable for the therapeutic purposes.

Data compiled in Table 4 showed that α-E and Vit. B1 are the most abundant fat and water soluble vitamins in *C. tiglium* seeds. Moreover, concentrations of Vit D3 and Vit B3 were too low to be detected. This was in accordance with Bello *et al*. [57] who mentioned during their study that the seeds could be good source of fat soluble vitamins.

HPLC Analysis of the free amino acids in the dried aqueous C. tiglium seeds extract

It was found that the dried aqueous *C. tiglium* seeds extract contains protein content representing about 25% (wt/wt) of the dried extract [38]. After isolation and purification processes, percent of the protein was about 23.5% (wt/wt) dried aqueous extract [37]. As presented in **Table 5**, there were eight essential and nine non-essential

TABLE 3. Percentages, physical and chemical characters of the prepared *C. tiglium* seeds extracts.

Character	*C. tiglium* seeds extracts		
	Ethyl alcohol	**Petroleum ether**	**Aqueous**
Percentage (%)	31	45	23
Physical characters:			
Color	Brown	Yellowish brown	Brown
Condition	Semi-solid	Oily	Semi-solid
Constituents:			
Carbohydrates	+	-	+
Flavonoids	+	-	+
Saponins	-	-	-
Tannins	-	-	-
Sterols	+	++	-
Alkaloids	+	-	+
Proteins	+	-	+
Anthraquinones	-	-	-

(-): Absent, (+): Present, (++): Appreciably present.

TABLE 4: Concentration of fat and water soluble vitamins in the dried powders of *C. tiglium* seeds.

Type of vitamin		Concentration (µg/g)
Fat soluble vitamins	Vit A	0.646
	Vit D3	ND
	α-E	**9.495**
Water soluble vitamins	B3	ND
	B1	**298.149**
	B6	0.288
	B9	0.695
	B2	1.328
	B12	0.479

ND: Not detected concentration.

amino acids identified in the dried aqueous *C. tiglium* seeds extract representing about 20.71% (wt/wt) and 79.29% (wt/wt) of the total amino acids, respectively. Moreover, it was found that isoleucine, methionine and phenyl alanine belong to the major essential amino acids representing about 4.74, 3.84 and 3.54% (wt/wt) of the total amino acids content, respectively. However, proline and aspartic acid belong to the major non-essential amino acid and represent about 14.41 and 13.32% (wt/wt) of the total amino acids content, respectively.

GLC analysis of mucilage after hydrolysis in the dried aqueous C. tiglium seeds extract

The carbohydrate content was estimated quantitatively in the dried aqueous *C. tiglium* seeds extract [25] and it was found that it represented about 16% (wt/wt) of the dried aqueous extract [31. Based on the analysis suggested by Evan and Matz [33, 34], it was noticed that no gelatinous precipitate appeared upon the reaction with potassium hydroxide. In addition, the red stain obtained as a result of the reaction with ruthenium red indicates the mucilaginous nature with

TABLE 5. HPLC Analysis of the free amino acids in the dried aqueous *C. tiglium* seeds extract.

Total amino acids			
Essential amino acids	**% of total amino acids**	**Non essential amino acids**	**% of total amino acids**
Threonine	1.59	Aspartic acid	13.32
Valine	2.42	Serine	11.30
Methionine	3.84	Glutamic acid	9.53
Isoleucine	4.74	Glycine	7.87
Leucine	1.63	Proline	14.41
Phenyl alanine	3.54	Alanine	4.02
Lysine	2.03	Tyrosine	10.85
Tryptophan	0.92	Histidine	4.74
		Arginine	3.25
Total essential amino acids	20.71	Total non essential amino acids	79.29

eliminating pectin. Furthermore, it was found the isolated mucilage expressed by 14.5% (wt/wt) of the dried aqueous extract. As compiled in **Table 6**, composition of the mucilage was determined qualitatively and quantitatively in the hydrolyzate by GLC.

GC/MS analysis of the saponifiable matter in the petroleum ether C. tiglium seeds extract

The lipoidal matter was quantified spectrophotometrically in the petroleum ether *C. tiglium* seeds extract [39]. It was found that the total steroidal and terpenoidal contents were expressed by 12% and 24 % of the dried extract as β-sitosterol and β-amyrin, respectively. Data compiled in **Table 7** showed the methylated ester derivatives of fatty acids that were identified in the saponifiable matter of the petroleum ether *C. tiglium* seeds extract by GC/MS. It was revealed that eighteen compounds were identified representing about 93.93% of the total saponifiable matter. The saturated fatty acids constituted about 13.68%, while the unsaturated fatty acids constituted 80.25%. Furthermore, it was found that 11,14-Octadecadienoic acid (26.57%) was the major unsaturated fatty acid, while Hexadecanoic acid (6.64%) was the major saturated fatty acid.

TABLE 6. GLC analysis of mucilage after hydrolysis in the dried aqueous *C. tiglium* seeds extract.

Authentic sugars	R.T (min.)	% of total mucilage hydrolyzate
Arabinose	8.31	23.38
Xylose	8.62	15.21
Ribose	8.97	11.84
Rhamnose	9.31	9.18
Galactose	13.29	13.15
Glucose	14.38	8.45
Total identified sugars		81.21

R.T: Retention time.

TABLE 7. GC/MS analysis of the saponifiable matter in the petroleum ether *C. tiglium* seeds extract.

Rt	%	Fatty acid methyl ester	Mol. weight	Base peak	Other fragments
14.79	5.04	9-Tetradecenoic acid	240	55	69, 74, 83, 110, 125, 137, 166, 208
15.43	1.05	3-Methyl-tetradecanoic acid	256	74	83, 101, 111, 129, 143, 156, 213, 225, 241
15.90	6.64	Hexadecanoic acid	270	74	87, 97, 129, 143, 199, 227, 239
17.64	1.04	3-Methyl-hexadecanoic acid	284	74	87, 101, 115, 157, 171, 213, 227, 241, 253, 270
19.73	8.10	9,11-Octadecadienoic acid	294	67	81, 95, 109, 123, 150, 164, 263
19.98	26.57	11,14-Octadecadienoic acid	294	67	81, 95, 109, 150, 164, 178, 263
20.09	13.84	9-Octadecenoic acid	296	55	69, 74, 83, 98, 111, 123, 137, 180, 222, 264
20.46	2.76	Octadecanoic acid	298	74	87, 97, 129, 143, 185, 199, 213, 255
21.84	1.06	Nonadecanoic acid	312	74	87, 97, 111, 129, 143, 157, 185, 199, 213, 269
22.00	2.04	5-Eicosenoic acid	324	55	67, 74, 96, 110, 123, 152, 180, 208, 250, 275, 292
22.77	3.06	11-Docosenoic acid	352	55	69, 74, 83, 97, 123, 152, 180, 208, 236, 263, 278, 292
23.24	0.09	Docosanoic acid	354	74	87, 97, 129, 143, 157, 199, 213, 255, 311, 323
23.95	4.26	15-Tetracosenoic acid	380	55	69, 74, 83, 97, 111, 123, 152, 194, 207, 222, 250, 264, 277, 291, 306, 348
24.31	5.09	16-Pentacosenoic acid	394	55	69, 74, 83, 97, 111, 125, 152, 194, 222, 236, 264, 278, 291, 320, 344, 362
24.68	1.04	24-Methyl-pentacosanoic acid	410	74	87, 97, 111, 129, 143, 157, 199, 213, 255, 269, 311, 325, 353, 367
26.13	6.05	5,9-Heptacosadienoic acid	420	81	95, 109, 141, 150, 164, 181, 207, 222, 278, 346, 371, 388
26.50	5.04	5,9-Octacosadienoic acid	434	81	95, 109, 141, 150, 163, 181, 207, 236, 264, 292, 319, 360, 385, 402
28.86	1.16	19-Octacosenoic acid	436	55	69, 74, 83, 97, 111, 125, 152, 194, 222, 236, 264, 292, 320, 362, 387, 406
Total saturated fatty acids: 13.68%					
Total monoenoic fatty acids: 34.49%					
Total dienoic fatty acids: 45.76%					
Total identified fatty acids: 93.93%					

Rt: Retention time

GC/MS analysis of the unsaponifiable matter in the petroleum ether C. tiglium seeds extract

Table 8 displayed components of the unsaponifiable matter in the petroleum ether *C. tiglium* seeds extract by GC/MS. It was found that nineteen compounds were identified representing about 80.35% of the total unsaponifiable matter. In addition, n-Docosane (14.42%) and 7-Phenyltridecane (9.10%) were expressed as major compounds of the unsaponifiable matter.

During the present study, four compounds were isolated from the petroleum ether *C. tiglium* seeds extract and purified several times by preparative TLC using different developing solvent systems. Consequently, these isolated compounds were characterized with recording their R_f values in benzene: ethyl acetate (8:2) accurately. All of the isolated compounds gave positive result with Lieberman-Burchard test indicating the presence of triterpenes and sterols [62]. Structures of these

TABLE 8. GC/MS analysis of the unsaponifiable matter in the petroleum ether *C. tiglium* seeds extract.

Rt	%	Compound	Mol. weight	Base peak	Other fragments
9.71	0.50	2,3-Dimethylnonane	156	57	43, 71, 83, 112, 141
11.96	0.61	n-Dodecane	170	57	43, 71, 85, 99, 113
18.78	0.97	2,6,11-Trimethyldodecane	212	57	43, 71, 85, 99, 127, 141,169
19.43	0.32	5-phenyl Decane	218	91	105, 119, 147, 161
21.23	0.70	2-phenyl Decane	218	105	119, 131, 181
21.87	1.42	6-phenyl undecane	232	91	105, 119, 161, 189
21.98	4.00	6-phenyl dodecane	246	91	105, 119, 147, 189
22.26	3.20	1-Hexadecanol	242	55	69, 83, 97, 111, 125, 196, 224
22.81	2.80	2-Phenyldodecane	246	105	119, 161, 207, 218
24.32	9.10	7-Phenyltridecane	260	91	105, 119, 133, 175, 189
24.74	5.89	2-Phenyltridecane	260	105	119, 147, 161, 190, 232, 245
25.28	4.75	1-Nonadecene	266	43	55, 83, 97, 111, 125, 139, 168
26.24	7.67	2-Methyl7-nonadecene	280	43	57, 69, 83, 97, 111, 125, 252
26.62	14.42	n-Docosane	310	43	57, 71, 85, 99, 113, 127, 239
26.77	6.88	1-Docosanol	326	43	55, 69, 83, 97, 111, 125
27.05	3.47	Ergosta-5,22-dien-3β-ol	398	398	199, 213, 255, 271, 300, 337, 355, 365
27.60	2.89	5α-Ergost-7-en-3β-ol	400	43	213, 255, 273, 327, 357
28.51	4.25	Stigmast-5-en-3β-ol	414	414	199, 213, 231, 241, 255, 273, 303, 329, 339, 367, 381, 396
39.48	6.51	17-Pentatriacontene	490	43	57, 83, 97, 111, 125, 139, 462
Saturated Hydrocarbons: 40.73% Unsaturated Hydrocarbons: 18.93% Fatty alcohols: 10.08% Sterols: 10.61% Total identified compounds: 80.35%					

Rt: Retention time

compounds have been elucidated by comparing their pectroscopical results with those published data.

Study of structure of the isolated compounds

Compound 1 (β sitosterol) (Fig. 1a): isolated from petroleum ether : chloroform (75:25) as white crystal, R_f 0.69, melting point 133-134 °C which in agreement with that reported in literature[63], **UVmax**: 203, **EI-MS** m/z (relative intensity): 414 (54%) for the molecular formula $C_{29}H_{50}O$, and base peak (100%) at *m/z* 43, in addition to other significant fragments at 369 (63%) corresponds to (M-45) due to loss of HO^+=CH-CH_3, also, 271 (22%) as a result of the formation of carbonation by β bond cleavage of side chain leading to the loss of $C_{10}H_{23}$ that corresponds to the M-143, beside to 396 (28%) due to the loss of a water molecule and 133 (18%), 105 (23%), 107 (26%), 95 (31%), 81 (34%), 69 (32%), 55 (41%).

The **IR** absorption spectrum (KBr, cm^{-1}) showed absorption peaks at 3379 (O-H stretch), 2943 and 2865 (C-H stretch), 1685 (Olefinic C=C), other absorption peaks were 1455 (Aromatic C=C Stretch), 1388 (Aromatic C-H Bending), 1036 (C-OH Stretch) and 885 (O-H Bending).

^{1}H-NMR (δ values, $CDCl_3$): 5.29 (1H,H-6) indicated the presence of an olefinic proton at C-6 owing to the double bond between C-5 and C-6, the presence of two singles at 0.66 (3H,s, Me-18) and1.12(3H,s,Me -19) refer to the angular methyl groups C-18 and C-19. Also the appearance of three doublets at δ 1.04(3H,d,Me-21),0.83(3H,d, *J*=6.8 Hz, Me-26), 0.80 (3H,d, *J*=6.4 Hz, Me-27), and one triplet at 0.86(3H,t, *J*=6.6 Hz,Me-29) confirm the presence of C-21, C-26, C-27, and C-29 methyl groups, respectively.

^{13}C-NMR: δ 11.89 (C-18), 11.98(C-29),

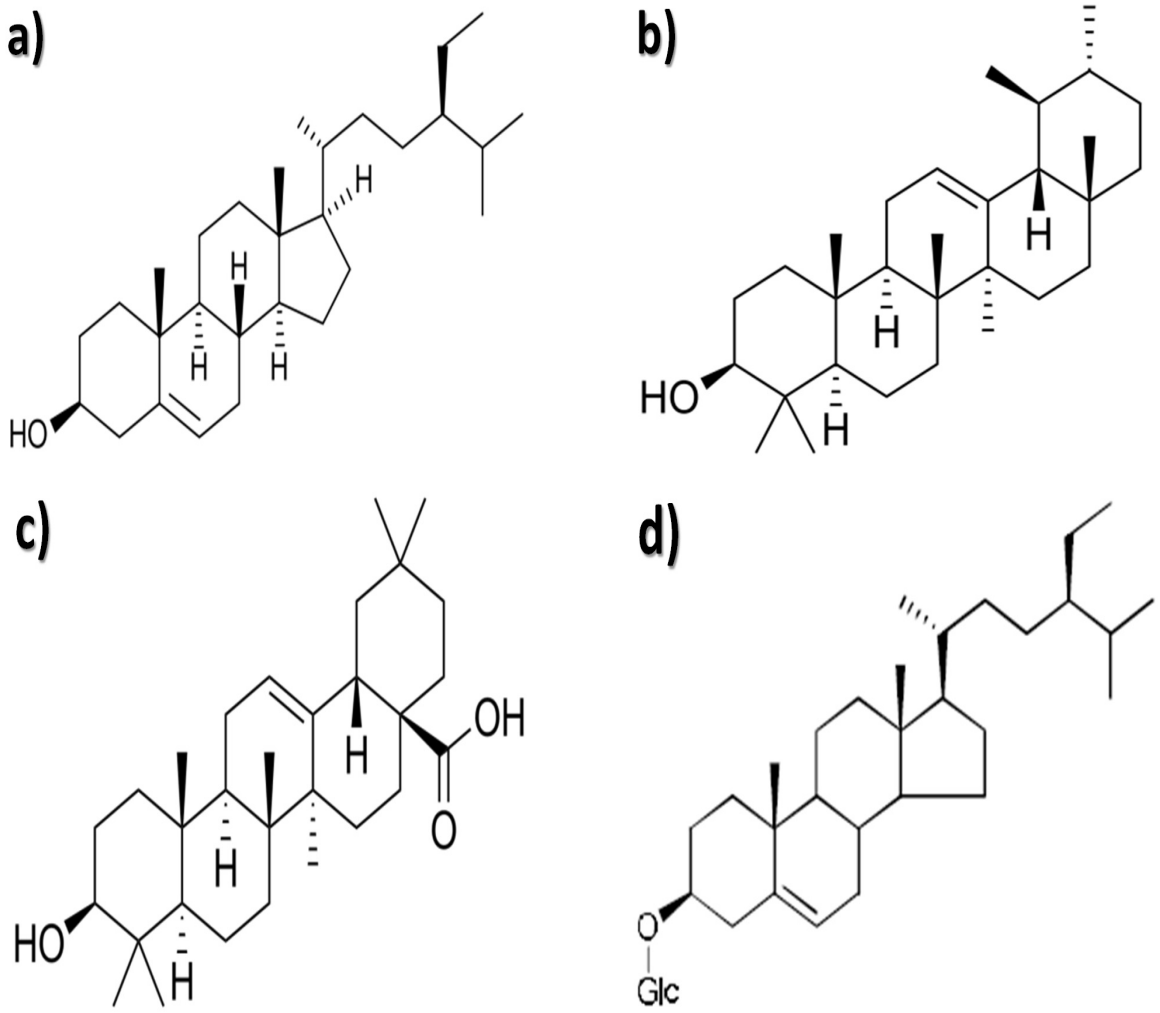

Fig. 1. Structure of the isolated compounds: a) β sitosterol, b) α-amyrin, c) Oleanolic acid and d) 3-O-β-D-glucopyranosyl-β-sitosterol .

18.69(C-21), 18.82(C-27), 19.34(C-19), 19.56(C-26), 21.42(C-11), 23.18(C-28), 24.35(C-15), 26.31(C-23), 28.36(C-16), 29.27(C-25), 31.64(C-2), 31.94(C-7), 32.17 (C-8), 32.48(C-22), 34.15(C-20), 36.35(C-10), 36.75(C-1), 37.45(C-4), 39.79(C-12), 42.36(C-13), 45.64(C-24), 50.13(C-9), 56.21(C-17), 56.72(C-14), 77.31(C-3), 121.46 (C-6), 140.72(C-5).

Compound 2 (α-amyrin) (Fig. 1b): isolated from petroleum ether : chloroform (50:50) as white needle crystals, R_f 0.66, melting point 185-186 °C as stated by Sira*et al*. [64]. **UV** data displayed characteristic absorption bands at 275, 260, 243 nm, **EI-MS** m/z (relative intensity) shows molecular ion at m/z 426 (30%) which is consistent with the molecular formula $C_{30}H_{50}O$ [65], base peak (100%) at m/z 218, in addition to other characteristic fragments at 43 (57%), 55 (43%), 57 (31%), 71 (23%), 95 (63%), 127 (24%), 175 (13%), 189 (11%), 203 (28%), 257 (9%), 293 (13%), 409 (7%), 411 (18%).

The **IR** spectrum (KBr, cm^{-1}) showed absorption peaks at 3438 (O-H Stretch), 3021, 2980, 2934, 2850, 2724 (C–H in conjugation), 1652 (ethylenic double bond), 1458 (aromatic C=C Stretch), 1372 (aromatic C-H Bending), 1124, 1021 (C-OH Stretch), 974, 886 (O-H bending).

^{1}H NMR (δ values, $CDCl_3$): 0.84 (s, 3H, $-CH_3$), 0.89 (d, 3H, $J = 7$ Hz, $-CH_3$), 1.06 (d, 3H, $J = 7.0$ Hz, $-CH_3$), indicating the triterpenoidal nature, 1.27–1.30 (d, 6H, $J = 7$ Hz, two secondary $-CH_3$) indicating the isopropenyl skeleton, 1.35 (s, 3H, $-CH_3$ at H-29) 1.49 (m, 2H, H-16), 1.53 (m, 2H, H-21), 1.59 (d, 1H, $J = 11.5$ Hz, H-18), 1.67 (s, 7 H, $-CH_3 \times 2$ and H-5), 1.78 (m, 1H, H-19), 1.80 (m, 2H, H-22), 1.89 (m, 2H, H-15), 2.02 (d, 2H, $J = 11.5$ Hz, H-1), 2.06 (s, 1H, H-9), 2.20-2.27 (d, 2H, $J = 11.5$ Hz, H-1 and H-16), 3.49 (s, 2H, H-7), 3.59 (m, 2H, H-2), 5.09 (s, 2H, H-11), 5.16 (s, 1H, H-12).

^{13}CNMR: δ 15.5 (C-24, C-25), 16.9 (C-26), 17.6 (C-29), 18.5 (C-6), 21.6 (C-30), 23.2 (C-27), 23.5 (C-11), 26.5 (C-16), 27.1 (C-15), 28.2 (C-23, C-28), 28.7 (C-2), 31.3 (C-21), 32.4 (C-7), 33.9 (C-17), 36.7 (C-10), 38.8 (C-1, C-4), 39.6 (C-19, C-20), 40.6 (C-8), 41.4 (C-22), 42.1 (C-14),47.8 (C-9), 55.2 (C-5), 59.2 (C-18), 79.7 (C-3), 124.6 (C-12), 139.4 (C-13).

Compound 3 (Oleanolic acid) (Fig. 1c): isolated from petroleum ether : chloroform (50:50) as white amorphous solid, R_f 0.62, melting point at 173 °C as stated in Galgon *et al*. [66].**UV max** at 205 nm, **EI-MS** m/z (relative intensity): 457 (43%) for the molecular formula $C_{30}H_{48}O_3$, and base peak (100%) at *m/z* 439 characteristic for pentacyclictriterpene skeleton with a 12-13 double bond, 411 (59%), 247 (61%), 203 (23%), 191 (14%), 177(24%).

The **IR** spectrum (KBr, cm^{-1}) showed absorption peaks at 3465 (O-H Stretch), 2957, 2855 (C-H Stretch), 1695(olefinic C=C), 1458 (Aromatic C=C Stretch), 1396, 1367, 1330 (Aromatic C-H Bending), 1184, 1161, 1030 (C-OH Stretch), 990, 865 (O-H bending).

^{1}H-NMR (δ values, $CDCl_3$): 0.77, 0.88, 0.89, 0.92, 0.96, 1.07 and 1.32 (7s, 21H, all $-CH_3$), 1.38 (m, 2H, H-21), 1.40 (m, 2H, H-16), 1.54 (m, 5H, H-18, H-19 and H-15), 2.11 (m, 3H, H-1 and H-9), 3.20 (t, 1H, $J = 7$ Hz, H-2), 3.41 (s, 2H, H-7), 4.55 (s, 2H, H-11), 4.62 (s, 1H, H-12). ^{1}H-NMR data showed the presence of an olefinic proton resonating at δ 4.62 (1H) (typical of oleane skeleton which was assigned to H-12).

^{13}CNMR: δ15.11 (C-25), 15.58 (C-24), 16.67 (C-26), 18.11 (C-6), 22.78 (C-11), 23.13 (C-30), 23.48 (C-16), 25.68 (C-27), 26.94 (C-2), 27.49 (C15), 28.01 (C-23), 30.47 (C-20), 32.24 (C-22), 32.54 (C-7), 32.97 (C-29), 33.69 (C-21), 37.01 (C-10), 37.89 (C-1), 38.52 (C-4), 39.24 (C-8), 41.02 (C-18), 42.13 (C-14), 45.76 (C-17), 46.23 (C-19), 47.85 (C-9), 54.79 (C-5), 79.34 (C-3), 121.89 (C-12), 144.51 (C-13), 182.03 (C-28). The presence of oleane skeleton was confirmed in the ^{13}C-NMR data with the signals in the region δ 15.11 ppm, at δ 121.89 and δ144.51 attributed respectively to seven methyl groups, to C-12, C-13 and 12–oleane skeleton.

Compound 4 (3-*O*-β-D-glucopyranosyl-β-sitosterol (daucosterol)) (Fig. 1d): isolated from petroleum ether : chloroform (25:75) as white crystals, R_f 0.58, melting point 281-282°C which in agreement with that stated byFaizi*et al*. [67].**UV max**: 205 and 197 nm. **EI-MS** m/z (relative intensity): 576 (36%) for the molecular formula $C_{35}H_{60}O_6$, and base peak (100%) at *m/z* 57, the other significant peaks were 414 (56%) corresponding to (M^+-glycosidic unit), 396 (47%) indicated the loss of a water molecule from *β*-sitosterol nucleus, beside 381 (34%), 329 (26%), 303(18%), and 275 (24%).

The **IR** absorption spectrum (KBr, cm^{-1}) showed absorption peaks at 3385 (O-H stretch), 2865 (C-H stretch), 1655 (olefinic C=C), other absorption peaks are 1452 (Aromatic C=C

Stretch), 1385 (Aromatic C-H Bending), 1055 (C-OH Stretch) and 892 (O-H bending).

^{1}H-NMR (δ values, $CDCl_3$): 5.34 (1H, br, H-6) for the anolefinic proton at C-6, 4.18 (1H, d,*J*=7.8 Hz, H-1'), 3.54 (1H, m, H-3), and 3.41(1H, d, H-2').

^{13}CNMR: δ ppm11.4 (C-18), 11.9 (C-29), 18.2 (C-21), 18.7 (C-27), 19.1 (C-19), 19.4 (C-26), 20.6 (C-11), 21.9 (C-28), 25.0 (C-15), 26.2 (C-23), 28.5 (C-16), 28.9 (C-2), 29.6 (C-25), 30.8 (C-7/8), 33.6 (C-22), 35.4 (C-20), 36.0 (C-10), 36.7 (C-1), 37.8 (C-4), 39.6 (C-12), 42.1 (C-13), 44.9 (C-24), 49.3 (C-9), 55.2 (C-17), 56.4 (C-14), 61.3 (C-6'), 71.2 (C-4'), 73.7 (C-2'), 76.9 (C-3), 77.4 (C-3'/5'), 100.6 (C-1'), 121.4 (C-6), 140.3 (C-5). The acid hydrolysis of the compound led to the presence of glucose in aqueous layer and β-sitosterol in the organic layer and by comparing with authentic reference samples in addition to the literature [68], the compound was identified as β-sitosterol-3-*O*-glucopyranoside.

Among the all noble metal nanoparticles, the Ag-NPs gained boundless interests due to their characteristic properties in addition to their significant antibacterial, antiviral, antifungal and anti-inflammatory effects. Moreover, silver exhibits vital functions as an antiseptic and displays a broad biocidal effect against various microorganisms through disruption of their unicellular membrane thus disturbing their enzymatic activities [69, 70]. Many reports have been published concerning synthesis of Ag-NPs using plant extracts revealed that the nanoparticulated extracts were economic, energy efficient and cost effective, in addition this technique provide healthier work places, communities, protecting human health and environment, leading to less waste and more safe products [71-73]. For this reason, during the current study, they can be incorporated into *C. tiglium* seeds extract to raise its antioxidant efficiency to be used for therapeutic purposes.

Assessment of the structure and morphology of fabricated nanomaterials

The XRD as well as TEM consider the most significant technique to examine structural properties of the fabricated nanomaterials. The Ag-NPs were prepared and studied using the XRD diffraction pattern. As revealed in Fig. 2a, it was noticed that XRD result of the prepared Ag-NPs displayed the characteristic peaks of metallic Ag^{o} found at 37.8°, 44.5° and 67.6° matching with the crystallographic planes (1 1 1), (0 0 2) and (0 2 2) of Ag-NPs, respectively and generates a typical of crystalline metallic Ag phase.

Also, XRD displayed separate diffraction peaks around 37.8°, which were indexed by the (002) of the cubic face-centered silver. These sharp Bragg peaks may as a result of capping agent using for stabilizing the prepared Ag-NPs. Strong Bragg reflections recommend that strong X-ray scattering centers in the crystalline phase and might be because of capping agents. An increase in the incubation time with Ag-NO_3 solution along with plant extract the synthesis of Ag-NPs increased. Presence of the plant extract reduced formation of $AgNO_3$ into Ag ions. Moreover, the secondary metabolites present in the extract act as a reducing and a capping agent for Ag-NPs synthesis [74-78].

In order to assess the shape, size and morphology of nanoparticles, TEM was used. It was revealed that the Ag-NPs were well dispersed and were predominantly spherical in shape, while some of the NPs were irregular in shape. Based on the obtained morphological data using TEM (Fig. 2b), it was demonstrated that the Ag-NPs was formed as a result of the chemical reduction which was carried out in presence of $AgNO_3$ solution. Presence of Ag nanoparticles maintained the homogeneity and uniformity of the distribution of Ag-NPs in the particles size range (5-10 nm) as revealed from TEM image. It was revealed that the Ag-NPs were well dispersed and were predominantly spherical in shape, while some of the NPs were irregular in shape**.** This was in agreement with findings of the experiment carried out by Ahmed and Ikram [79]. Furthermore, evidence for the formation of Ag-NPs prepared within the chemical reduction method was demonstrated using UV-visible spectroscopy. This technique is a precise suitable and reliable method for the main characterization of manufactured nanoparticles which is correspondingly used to display the Fabrication as well as stability of Ag-NPs.

The Ag-NPs have exceptional optical properties that generate strongly interrelate with exact wavelengths of light. Also, UV-Vis spectroscopy is easy, fast, simple and discerning for various types of NPs, requests simply a short period time for measurement. As illustrated in Fig. 2c, the prepared Ag-NPs showed a sharp peak at 450 nm that reveals the formation of Ag-NPs. Consequently, DLS is mostly used to determine

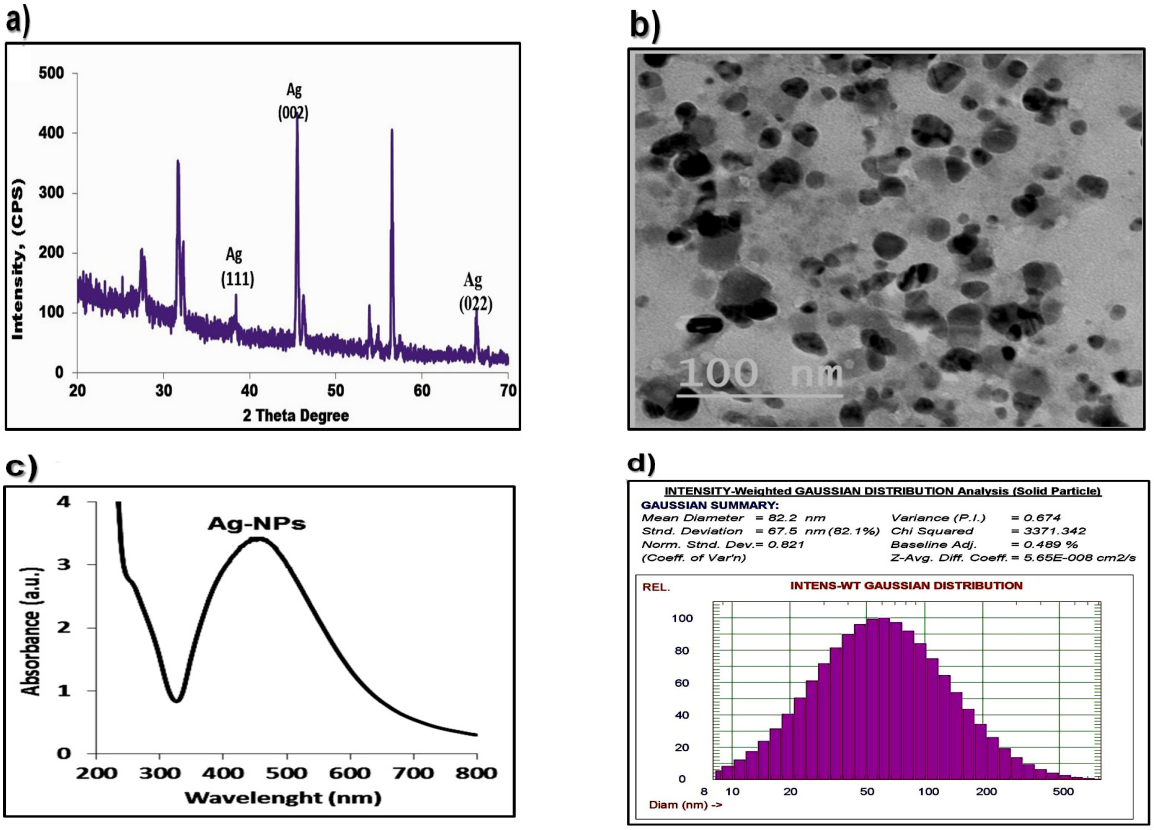

Fig. 2. a) XRD pattern of the prepared Ag-NPs, b) TEM image of Ag-NPs, c) UV-spectroscopy of the prepared Ag-NPs and d) DLS of the Ag-NPs.

particle size as well as size distributions in aqueous solutions. The size attained from DLS is frequently larger than that particle size obtained from TEM, which might be because of the effect of Brownian motion. DLS is a nondestructive technique used to acquire the average diameter of the prepared dispersed nanoparticles in aqueous solutions. As presented in Fig. 2d, it was found that the particle size distribution of the fabricated Ag-NPs has main diameter around 82 nm.

The plants were effectively involved in synthesis and controlled formation of Ag-NPs. The nanoparticles size differs from TEM to DLS and this may be the reason of particles agglomeration. Furthermore, the nanoparticles exhibited good stability and this might be due to presence of secondary metabolites as a capping or reducing agent [80].

Total antioxidant capacity and free radical scavenging activity in different C. tiglium seeds extracts before and after incorporating Ag-NPs.

Polyphenols are the most common biologically active molecules. They are structurally characterized by presence of one or more phenol units. They are considered as one of the most important classes of secondary plant metabolites that play an important role in prevention of chronic diseases owing to their antioxidants potentials [81 & 82]. As illustrated in Table 9, it was found that the aqueous *C. tiglium* seeds extract contains the highest concentration of polyphenolic compounds (699.21± 6.93 mg gallic acid/100 gm) as compared to the other extracts. This was in agreement with the study suggested by Salatino et al. [52] who reported that these phenolic compounds were represented mainly by flavonoids, lignoids and proanthocyanidins predominate. It was shown that the nanoparticulated extracts exhibited elevated concentrations on total polyphenolic compounds in ethanolic, petroleum ether and aqueous *C. tiglium* seeds extracts (873.54 ± 9.31, 485.91 ± 5.18 and 962.05 ± 10.25 mg gallic acid/100 gm, respectively) than the crude extracts (617.87 ± 3.85, 388.24 ± 6.47 and 699.21 ± 6.93 mg gallic acid/100 gm, respectively). This was in accordance with Abdelhady and Badr [83] who reported that incorporation of Ag-NPs increased concentration of the polyphenolic compounds with respect to the native extract (without Ag-NPs).

In addition, it was noticed that the aqueous *C. tiglium* seeds extract proved promising antioxidant capacity, total reducing power and free radical scavenging activity by 0.856 ± 0.019 mg gallic acid / gm, 8.81 ± 1.10 and 73 %, respectively. The antioxidant properties of the seeds extract might be attributed to presence of the main components of the antioxidant fractions (α-bisabolol, α- eudesmol and guaiol) [84]. In addition, the aromatic acids (vanillic and 4-hydroxy-benzoic acid along with N-methyltyrosine) have shown remarkable antioxidant activity [85]. Furthermore, it was noticed that incorporation of Ag-NPs enhanced the antioxidant properties through increasing the total antioxidant capacity, total reducing power and free radical scavenging activity in comparison with the native extracts. This was in accordance with Abdelhady and Badr [83] who suggested that nanoparticulated extracts increased levels of the active phytoconstituents which exhibit more antioxidant potentials and possesses significant free radical scavenging activity than the crude extracts.

The *in vitro* anticancer activity showed that the seeds powder and ethanol extract exhibited equal anticancer activity against human colon cancer cells with IC_{50} 36.3 µg/mL). This was in accordance with Mohd Ali et al. [86] who reported that the ethanolic *Croton* extracts exhibited high antioxidant activity by means of DPPH radical scavenging activity, reducing power and total antioxidant capacity. Furthermore, this might be due to the highest total phenolic and total flavonoid content, thus suggesting the potential use of *C. tiglium* seeds extract as a natural source of antioxidant. Petroleum ether extract showed no anticancer activity. This might refer to presence of the essential oils that have limited cytotoxicity against colon carcinoma [87]. While the aqueous extract showed anticancer activity with IC_{50} 33.9 µg/mL. This might be attributed to presence of wide range of the phytochemical constituents that can inhibit the process of carcinogenesis effectively and prevent the development of invasive cancer [88, 89]. As suggested byAbdelhady and Badr [83], incorporation of Ag-NPs into the different extracts increased the cytotoxicity against growth of human colon cancer cells compared to the crude ones. This was manifested by their reduced IC_{50} for different used cell lines. This attributed to enhancement of total polyphenolic compounds, the total antioxidant capacity, iron reducing

TABLE 9. Total antioxidant capacity and free radical scavenging activity in different *C. tiglium* seeds extracts before and after incorporating Ag-NPs.

Solvent		Total antioxidant capacity (mg gallic acid/gm)	Total polyphenols (mg gallic acid/100 gm)	Reducing power (µg/mL)	ABTS (mg/gm)	Antioxidant activity (%)	IC_{50} (µg/mL)
Seeds		0.570 ± 0.014	487.18 ± 9.40	7.25 ± 0.91	22.521	62 %	36.3
Ethanol	Before	0.444 ± 0.002	617.87 ± 3.85	3.30 ± 0.42	21.205	53 %	36.3
	After	0.60 ± 0.04	873.54 ± 9.31	7.06 ± 0.88	29.45	71 %	18.75
P. ether	Before	0.217 ± 0.009	388.24 ± 6.47	0.37 ± 0.08	0.838	22.2 %	50
	After	0.36 ± 0.02	485.91 ± 5.18	4.31 ± 0.54	1.25	35.7 %	23
Aqueous	Before	**0.856 ± 0.019***	**699.21 ± 6.93***	**8.81 ± 1.10***	**41.525***	**73 %***	**33.9***
	After	**1.08 ± 0.06***	**962.05 ± 10.25***	**14.21 ± 1.78***	**62.74***	**87 %***	**3.13***

*: The most effective extract as compared to the others, Value expressed as mean ± SE of four replicates

power and free radicals scavenging activity and hence increasing the anticancer activity through lowering growth of the cancer cells.

Incorporation of Ag-NPs into the extract caused no toxicity in the experimental animals when administrated orally by stomach tube. This was in accordance with the concept reported by Mohanpuria et al. [90] who suggested that green route synthesis of Ag-NPs found with less toxicity. This was supported by our findings that emphasized that the LD_{50} of the ethanolic seeds extract-Ag nanocomposite was about7.95 ml/ Kgandthe therapeutic dose was about 0.8 ml/ Kg. In the petroleum ether seeds extract-Ag nanocomposite, the LD_{50} was about 5.2 ml/Kg and hence the therapeutic dose was 0.52 ml/Kg. Moreover, the aqueous *C. tiglium* seeds extract-Ag nanocomposite was found with LD_{50} about 65 ml/Kg and hence the therapeutic dose was about 6.5 ml/Kg.

Conclusions

In this study, it was concluded that *C. tiglium* seeds contain various active phytoconstituents such as carbohydrates (glycosides), flavonoids, sterols (triterpenes), alkaloids (nitrogenous compounds) and proteins. Incorporation of Ag-NPs into the different extracts (ethanolic, petroleum ether and aqueous seeds extracts) enhanced the antioxidant properties through increasing the total antioxidant capacity, total reducing power and free radical scavenging activity in comparison with the crude extracts. Therefore, it was shown that the nanoparticulated extracts exhibited elevated cytotoxicity against growth of human colon cancer cells compared to the crude ones. No toxicity occurred as a result of incorporation of Ag-NPs into the extract when administrated orally by the experimental animals.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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تقييم الكفاءة المضادة للأكسدة لمستخلصات بذور نبات حب الملوك بعد دمج جزيئات الفضة النانومترية

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هدفت هذه الدراسة إلى زيادة كفاءة مستخلصات بذور نبات حب الملوك (*C. tiglium*) عن طريق دمج جزيئات الفضة النانومترية (Ag-NPs) من خلال رفع درجة القدرة ضد نمو خلايا سرطان القولون. ومن خلال هذه الدراسة وجد أن بذور نبات حب الملوك يحتوي على مكونات متنوعة على سبيل المثال، الكربوهيدرات (جليكوسيدات) ، فلافينويدات، تربينات ثلاثية، ستيرولات، قلويدات وبروتينات. وقد تم عمل تحلل للبروتينات في مستخلص البذور المائية المجففة إلى أحماض أمينية حرة، أحماض أمينية أساسية (٢٠,٧١٪) وأحماض أمينية غير أساسية (٧٩,٢٩٪). وعلاوة على ذلك، وجد أن نسبة السكريات الكلية التي تم تحديدها والتعرف عليها تمثل حوالي ٨١,٢١٪ من إجمالي نسبة الكربوهيدرات التي تم تحللها وتحليلها في مستخلص البذور المائية المجففة. وأوضحت التجربة بعد عملية التصبن وتحليل المحتوي الدهني في مستخلص الإيثير البترولي أنه يحتوي على أحماض دهنية مشبعة (١٣,٦٨٪)، أحماض دهنية أحادية الكربوكسيل (٣٤,٤٩٪)، أحماض الدهنية ثنائية الكربوكسيل (٤٥,٧٦٪) وأحماض دهنية محددة ومتعارف عليها (٩٣,٩٣٪). أما المادة الغير قابلة لعملية التصبن، وجد أنها تتمثل في الهيدروكربونات المشبعة (٤٠,٧٣ ٪)، والهيدروكربونات غير المشبعة (١٨,٩٣٪)، كحولات دهنية (١٠,٠٨٪)، ستيرولات (١٠,٦١٪) والمركبات المحددة المتعارف عليها سابقا (٨٠,٣٥٪). بالإضافة إلى ذلك، تم عزل والتعرف على أربعة مركبات من مستخلص الإيثير البترولي (β sitosterol, α-amyrin, Oleanolic acid and ٣-*O*-β-D-glucopyranosyl-β-sitosterol (daucosterol)).

وجد أن دمج جزيئات الفضة النانومترية (Ag-NPs) في أي من هذه المستخلصات لم يكن له أي سمية على حيوانات التجارب عند تناوله عن طريق الفم. وأن الجرعة المميتة المتوسطة (LD_{50}) من مستخلصات الإيثانول، الإيثير البترولي والمائية المدمجة بجسيمات الفضة النانومترية كانت ٧,٩٥، ٥,٢ و٦٥ مل / كجم على التوالي.