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Comparative study of phenolic content, antioxidant potentials and cytotoxic activity of the crude and green synthesized silver nanoparticles′ extracts of two *Phlomis* species growing in Egypt

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Abstract
Bio-green method for synthesis of nanoparticles is advantageous compared to chemical and physical methods by virtue of being ecological friend and of comparable lower cost, the present study aimed the synthesis of silver nanoparticles for two *Phlomis* species (*P. aurea* Decne and *P. floccosa* D.) crude methanol extracts to give their nanoparticles' extracts (PAAgNPs and PFAgNPs), they were characterized using UV/Vis spectrophotometer, Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) where formation and stability of the reduced silver nanoparticles in colloidal solution was monitored by UV/Vis spectrophotometer, the gained nanoparticles were more predominantly spherical in shape with an average particle size of 30 nm.
Green synthesized nanoparticles' extracts (PAAgNPs and PFAgNPs) showed more total phenolic compounds (64.96±2.90, 62.40±2.85 & 51.73±2.75, 49.33±2.71), flavonoid (32.16±2.05, 29.43±1.95 & 29.50±2.25, 27.58±2.14), phenylethanoid glycosides (21.40±1.51, 22.85±1.92 & 18.25±1.46, 18.60±1.25) and iridoid (11.03±1.02, 10.15±0.88 & 9.40±0.95, 8.65± 0.72) contents for *P. aurea* Decne and *P. floccosa* D. respectively.
The nanoparticles' extracts exhibited enhanced antioxidant potentials in dose dependent manner at two dose levels 50 and 100 µg ml^{-1} against stable DPPH radical (2, 2- diphenyl-1-picrylhydrazyl) using butyl hydroxyl toluene (BHT) as a reference standard, the gained results were as follows; 67.83 ± 2.77, 62.05 ± 3.96 & 55.45 ± 2.52, 49.24 ±2.65 and 95.50 ± 1.84, 93.05 ± 1.75& 88.44 ± 3.90, 84.45 ± 1.51 for *P. aurea* Decne and *P. floccosa* D. respectively, moreover, the assessment of cytotoxic activity towards three cell lines namely HEP-G2**,** HCT-116 and MCV-7 using Cisplatin as a reference anticancer drug reported the significant selective reduction of IC_{50} of the nanoparticles' extracts compared to the crude methanol extracts.
The overall results revealed that elevation of the level of different constituents in the nanoparticulated extracts was correlated to significant increase in their antioxidant potentials and cytotoxic activity which suggested the possible involvement of the phytochemical constituents in the prepared silver nanoparticle.

Keywords: Nanoparticles, *Phlomis aurea* Decne, *Phlomis floccosa* D., total phenolic, flavonoids, phenylethanoids, iridoid, antioxidant and cytotoxic

Introduction
Currently nanotechnology is a great application tool for exploring the darkest avenues of medical sciences in several prospects as imaging [1], targeting drug delivery [2] and artificial implants [3] where the inorganic metal nanoparticles can fight against human pathogens [4] and dangerous diseases [5].
The thumb challenge in nanotechnology is for the development of efficient and green chemistry involved experimental protocols for the synthesis of nanomaterial for a required size, shape and dispersivity [6] where silver nanoparticles have unique optical, electrical, and thermal properties that play an indispensable role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants and tissue engineering [7], they are used in the development of new technologies in electronics material science and medicine areas and due to their extensive applications more researches are being conducted on the silver nanoparticles by the scientists throughout the world [8].
Synthesis of silver nanoparticles using non-biodegradable compounds as reducing agents is potentially hazardous to the environment and biological systems compared to biological methods which are considered as safe, environmental friend, cost effective and of significant competitive feasibility [9, 10].
Genus *Phlomis* L. (Lamiaceae), comprises more than 100 species of herbaceous plants, subshrubs and shrubs native from Mediterranean region east across central Asia to China [11-13],

plants belonging to this genus have been used in traditional medicine as stimulants, tonics, respiratory tract disinfectants and wound healers [14] meanwhile, different species were verified to exhibit some biological activities as anti-inflammatory, anti-nociceptive, anti-mutagenic, anti-allergic, antimalarial, antibacterial, antifungal, antiparasitic and cytotoxic [15-20], as well as extending lifespan in various heterotrophic organisms and longevity-defining cellular processes by virtue of existence of kaempferol and caffeoyl phenylethanoid glycoside contents [21].

Phytochemical studies of several *Phlomis* species revealed the existence of lignans, neolignans, alkaloids, terpenoids, iridoids, flavonoids, phenolic compounds like phenyl-propanoids, phenylethanoids, as well as their glycoside derivatives [22-24].

P. aurea Decne and *P. floccosa* D. are being used in folk medicine as antidiabetic [25] and for honey production [26] respectively.

Phytochemical studies of *P. aurea* Decne revealed the existence of the flavonoids 7-rutinosides,7-*p*-coumaroyl glucosides of naringenin, apigenin, luteolin and chrysoeriol, hispidulin 7-glucoside, luteolin 7-diglucoside, vicenin-2, lucenin-2, chrysoeriol-7-O-β-glucopyranoside, acacetin-7-O-β-glucopyranoside, luteolin-7-O-β-glucopyranoside; iridoids 3-epiphlomurin, phlomurin, auroside, lamiide, 8-epiloganin, ipolamiide, in addition to megastigmane glucoside "phlomuroside", benzyl alcohol glycoside "benzyl alcohol-O-β-xylopyranosyl-(1 ⟶ 2)-β-glucopyranoside", phenol glycosides acteoside (verbascoside), phenylethanoid glycoside 2-phenylethyl-O-β-xylopyranosyl-(1 ⟶ 2)-β-glucopyranoside and the lignan liriodendrin [26-29] meanwhile, *P. floccosa* D. showed similar flavonoid pattern, but with no flavanones [27], in addition to two iridoid glycosides [30].

Materials and Methods

Plant material: Shrubs of *Phlomis aurea* Decne and *P. floccosa* D. were collected during their flowering and fruiting stage from Saint Catherine (Wadi Gabal), South Sinai (July 2015) and El-Omayed Protectorate, Matrouh Governorate (August 2015) Egypt respectively, their identities were established by Prof. Dr. Abdo Marey, Prof. of Botany, Faculty of Science, Al-Azhar University. Voucher specimens were deposited in a herbarium in department of Pharmacognosy, Faculty of Pharmacy, Al Azhar University, Cairo, Egypt. Shrubs collected were cleaned, air-dried, powdered and kept in tightly closed amber coloured glass containers protected from light at low temperature.

Material for synthesis of nanoparticles: Silver nitrate (Sigma Chemical Co., St. Louis, MO, USA).

Material for determination of total phenolic content: Folin-Ciocalteu reagent (Sigma Chemical Co., St. Louis, MO, USA), and Gallic acid (E. Merck, Darmstadt, Germany).

Material for determination of total flavonoid content: Quercetin (Merck Co. Darmstadt, Germany) and Aluminium chloride (E. Merck, Darmstadt, Germany).

Material for determination of total phenylethanoid glycosides′ content: Arnow′s reagent [31] and Verbascoside (Sigma-Aldrich Quimica South Madrid Spain).

Material for determination of total iridoid content: Trim and Hill reagent [32] and Herbagoside (Sigma-Aldrich Quimica South Madrid, Spain).

Material for determination of antioxidant effect: DPPH (Sigma-Aldrich Quimica, South Madrid, Spain), Silica gel 60 F_{254} (Merck, Darmstadt, Germany), Mobile phase [butanol: acetic acid: water (40: 10: 50)] and Butylated hydroxyl toluene (BHT): Sigma-Aldrich, Quimica, South Madrid, Spain.

Material for determination of anticancer effect: Hepatocellular carcinoma cells (HEP-G2), Colon carcinoma cells (HCT-116) and Breast carcinoma cells (MCF-7), they were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA), they were grown on Roswell Park Memorial Institute (RPMI) 1640 medium (Nissui Pharm. Co., Ltd., Tokyo, Japan) and were supplied through The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Apparatus: Soxhlet, vacuum oven (Vacucell, Einrichtungen GmbH), Genesys Spectrophotometer (Milton Roy, INC., Rochester, NY) for UV/Vis. Investigation of nanoparticles, field emission scanning electron microscope (SEM, JSM 6490A, Jeol, Tokyo, Japan), Chromatographic glass jars, 96 Micro-well™ Plates, Conical Wells, Spectrophotometer (Perkin-Elmer Lambada 3) for quantitative determination of antioxidant effect, Rotatory evaporator (BUCHI Rotavapor® R-210/R-215, Germany), Genesys Spectrophotometer (Milton Roy, INC., Rochester, NY) for quantitative estimation of total phenolics, flavonoids, phenylethanoid glycosides & iridoids and Centrifuge.

Preparation of extracts: 50g of each dried powdered plants under investigation were extracted separately by soxhlet for 24h with methanol, after filtration, extracts were concentrated under vacuum then washed within *n*-hexane until the chlorophyll was completely removed; the washed methanol extracts were filtered and used for study.

Preparation of silver nanoparticles: *Phlomis aurea* Decne and *Phlomis floccosa* D. methanol extracts were used to produce silver nanoparticles where they were dried in vacuum oven at 40 ºC, the collected crude methanol extract was used for nanoparticle synthesis.

Silver nitrate ($AgNO_3$) was used as a source of metal for synthesis of nanoparticles where 1% aqueous solution of individual extracts were mixed with $AgNO_3$ solution in 0.1% in (1:1) ratio, they were vigorously mixed and incubated at room temperature for 3h where reduction process is followed by on immediate change from yellowish to brownish colour in the reaction vessel indicating the formation of PAAgNPs and PFAgNPs respectively, the produced AgNPs exhibit this brownish colour in solutions due to excitation of their surface plasmon resonance, the change in colour was observed by naked eye first and subsequently was analyzed by UV/Vis. Spectroscopy.

The obtained nanoparticles PAAgNPs and PFAgNPs were purified through centrifugation at 10,000 rpm for 5 min, washed and dried in vacuum chamber for 24 h at 35 ºC [33].

Characterization of silver nanoparticles: Formation of the silver nanoparticles (PAAgNPs and PFAgNPs) was monitored with the help of UV-Vis. Spectroscopy, their shape and size

were determined using field emission scanning electron microscope (TEM), they were subsequently characterized to record the localized surface plasmon resonance of sliver nanoparticles at 200-800 cm^{-1}. The size and morphology was examined using Scanning electronic Microscopy (SEM) and Transmission Electron Microscopy (TEM).

Determination of the total phenolic compounds: The concentration of total phenolic compounds in methanol and nanoparticulated extracts were determined spectrophotometrically using the Folin-Ciocalteu reagent which is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic compounds and polyphenol antioxidants [34]. Standard curve was done using different concentrations of gallic acid (10:60 µg/ml) in methanol, the concentrated extracts of the tested plants were dissolved each in least methanol volume then completed to 10ml, 100µl of these extracts were separately diluted with 8ml distilled water, to each sample 0.5ml of 50% Folin-Ciocalteu reagent was added and left 8 min, then 1.5 ml of 5% sodium carbonate was added, mixed and allowed to stand for 60 min. protected from light. Their absorbance was measured at 725 nm using methanol as blank and the concentration of the total phenolic content of each extract was calculated.

Determination of total flavonoids: Determination of the total flavonoid content in methanol and nanoparticulated extracts was done colourimetrically using aluminum chloride solution [35]. Standard curve was done using different concentrations of quercetin in methanol (six serial 2 fold dilution to give 120-20 µg /ml). 100µl of each extract (previously prepared) were added to a 96 Micro-well plate and then 100µl of 2% aluminum chloride solution in methanol, after 10 min, their absorbance was measured at 415 nm using methanol as blank and the concentration of total flavonoids in each extract was calculated.

Determination of the total phenylethanoid glycosides: The total phenylethanoid glycosides′ content in methanol and nanoparticulated extracts were done colourimetrically by using Arnow's reagent which is a mixture of 0.5M hydrochloric acid, 10% (w/v) of sodium nitrite and 10% (w/v) of sodium molybdate (4:1:4) [31]. Standard curve was done using different concentrations of standard verbascoside in methanol (six serial 2 fold dilution to give 180-100µg /ml), 1ml of the each extract dissolved in methanol (400µl/ml) was added to 2 ml of Arnow′s reagent and 2 ml of 2 M sodium hydroxide solution, then the solution was adjusted to 10ml with distilled water where production of purple colour indicates the presence of phenylethanoid glycosides, after 10 min the absorbance was measured at 525 nm using methanol as blank. The concentration of the total phenylethanoid glycosides in each extract was calculated.

Determination of the total iridoids: Determination of the total iridoid content in methanol and nanoparticulated extracts was done colourimetrically using Trim and Hill reagent which is a mixture of acetic acid, 0.2% copper sulfate and hydrochloric acid (10:1:0.5) [32]. Standard curve was done using different concentrations of standard herbagoside in methanol (six serial 2 fold dilution to give 120-220 µg/ml), 1ml of the plant extract dissolved in methanol (400µl/ml) was mixed with 4 ml of Trim and Hill reagent, and then heated at 110 ºC for 5 min where production of blue colour indicates the presence of iridoids, then the absorbance was measured at 609 nm using methanol as blank, concentration of total iridoids in each extract was calculated.

Determination of antioxidant effect: Determination of the antioxidant effect of methanol and nanoparticulated extracts was done according to stable DPPH radical technique both qualitatively using thin layer chromatography (TLC) and quantitatively using spectrophotometric method.

a. TLC assay: This assay was performed according to Cavin *et al.,* [36] where 20µl aliquot of each tested extract was spotted on silica gel plates and developed using butanol: acetic acid: water (40:10:50) as a mobile phase, after development, the dried TLC plates were sprayed with 0.2% DPPH solution in methanol and examined after 30 min. active antioxidants compounds appeared as yellow spots against purple background.

b. Spectrophotometric assay: This assay was performed according to Gialvez *et al.,* [37] where the test was carried out on 96 Micro-well plate, standard curve was done using different concentrations of BHT (butylated hydroxytoluene) in methanol (7 serial 2 fold dilutions to give final range of 60 to 10µg/ml), 50µl of a 0.022% DPPH solution in methanol was added to a range solution of different concentrations (7 serial 3 fold solutions to give final range of 1000 to 1.3µg /ml) of each of the tested extracts in methanol (230µl) and their absorbance was measured at 515 nm after 30 min.

***In vitro* screening of the cytotoxic activity:** The cytotoxic activity of methanol and nanoparticulated extracts was screened against three human tumor cell lines, namely hepatocellular carcinoma cells (HEP-G2), colon carcinoma cells (HCT-116) and breast carcinoma cells (MCF-7), the cells were supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin, they were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were sub-cultured two to three times a week.

Cytotoxic activity was determined by using cell viability assay method [38], the cells were plated in a 96-multiwell plate (104 cells/ well), for 24 h, before treatment with the extracts to allow attachment of cells to the wall of the plate. Different concentrations of the tested extracts 0.780, 1.560, 3.125, 6.250, 12.500, 25.00, 50.00 and 100.00 µg/ml in DMSO) were added to the cell monolayer; triplicate wells were prepared for each concentration, monolayer cells were incubated with the tested samples for 48 h at 37 °C, in an atmosphere of 5% CO_2, after 48 h, the cells were fixed, washed and stained with sulforhodamine-B stain, the excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. The colour intensity produced was measured in an ELISA reader where the relation between surviving fraction and the extracts′ concentration is plotted to get the survival curve of each tumor cell line after treatment with screened extracts.

Cisplatin was used as a reference drug, data fitting and graphics were performed by means of the Prism 3.1 computer program (Graph Pad soft-ware, USA), in addition, concentration-response curves were prepared and the IC_{50} values were determined.

Screening of the cytotoxic activity of the tested extracts was carried out in The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Statistical analysis: The statistical analysis of the outcome data was carried out using one way analysis of variance (ANOVA) followed by student t-test, P value <0.05 were considered as significant [39].

Result and Discussion

Nanotechnology is an important field of science dealing with synthesis and manipulation of particles of average size range (10 to 100 nm), in this size range all the physical, chemical and biological properties change in fundamental ways for both individual atoms/molecules and their corresponding bulk [40]. Nowadays metallic nanoparticles are being applied in pharmaceutical field, the strategy includes production of nanoparticulated material, then investigation or utilization of their mysterious physicochemical and optoelectronic properties [41].

Fig 1: Morphology of *Phlomis aurea* Decne and *Phlomis floccosa* D.

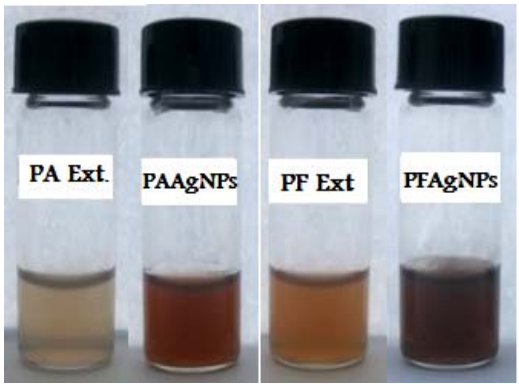

Fig 2: Color changes when crude methanol extracts of plant were mixed with AgNO3 solution.

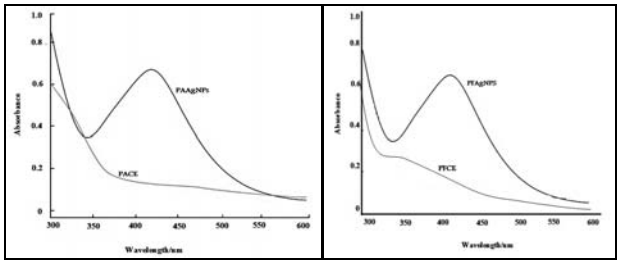

Fig 3: UV/Vis spectra of crude extracts (PACE&PFCE) and silver nanoparticles (PAAgNPs& PFAgNPs).

Among the all noble metal nanoparticles, silver nanoparticle gained boundless interests because of their unique properties as chemical stability and good conductivity, in addition they exhibit significant antibacterial, antiviral, antifungal, anti-inflammatory effects, moreover; they are used in cancer diagnosis and treatment. They can be incorporated into pharmaceutical products as cosmetics, wound dressings, topical creams and antiseptic sprays where silver functions as an antiseptic and displays a broad biocidal effect against various microorganisms through disruption of their unicellular membrane thus disturbing their enzymatic activities [42-44].

Many reports have been published concerning synthesis of silver nanoparticles 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 [20, 45-49].

P. aurea Decne and *P. floccosa* D. crude methanol extracts (PACE & PFCE) were employed for green synthesis of their nanoparticulated extracts (PAAgNPs & PFAgNPs) where they were added to silver nitrate solution, after 24h the colour of the reaction mixture changed to dark brown indicating the formation of silver nanoparticles (Figures 1, 2). The crude and nanoparticulated extracts were subjected UV-Vis. Spectro-photometric analysis to detect the formation and stability of produced metal nanoparticles in the reaction mixture. The UV-Vis. spectra recorded exhibited maximum absorption of nanoparticles at a wavelength of 430 & 420 nm for PAAgNPs & PFAgNPs respectively, these peaks corresponded to the surface plasmon resonance of the synthesized silver nanoparticles as their absorbance measurements lies in the range of 450–500 nm [50, 51] (Figure 3).

SEM analysis was employed to determine the surface morphology and the topography of synthesized silver nanoparticles where the size of silver nanoparticles ranged from 27 to 33 nm, with an average size 30 nm, the gained SEM images showed that the gained silver nanoparticles were mostly spherical in shape while TEM analysis revealed that most particles were obviously spherical in shape and well dispersed, with an average size around 30 nm (Figure 4).

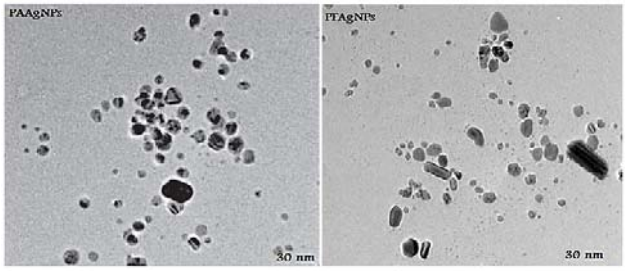

Fig 4: TEM micrograph of silver nanoparticles synthesized by using silver nanoparticulated extracts of *Phlomis aurea* Decne and *Phlomis floccose* D. (PAAgNPs & PFAgNPs).

Oxidative stress is the imbalance between protective systems and the production of free radicals caused by oxidative stress, the excess of reactive species can damage cell lipids, proteins and DNA which might result in loss of function and even cellular death leading to many diseases as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders [52].

Polyphenols represent a group of biologically active molecules which is common in plants and is structurally characterized by the presence of one or more phenol units, they constitute one of the most important classes of secondary plant metabolites those play important roles in prevention of chronic diseases owing to their antioxidants potentials [52-54].

Quantitative estimation of the total phenolic content of the

investigated extracts of using Folin-Ciocalteu reagent showed that the nanoparticulated extracts exhibited elevated levels (64.96 ± 2.90 & 62.40 ± 2.85 mg %) than the crude extracts (51.73 ± 2.75 & 49.33 ± 2.71 mg %) of *Phlomis aurea* Decne and *Phlomis floccose* D. respectively (table 1), the total phenols were measured by in terms of gallic acid equivalent (standard curve equation: y=0.05x±0.0545, r^2=0.9873) (Figure 4); while, quantitative estimation of total flavonoids using aluminum chloride reagent and quercetin as standard revealed that nanoparticulated extracts exhibited elevated levels (32.16 ± 2.05& 29.50 ± 2.25 mg %) than the crude extracts (29.43 ± 1.95 & 27.58 ± 2.14mg %) of *Phlomis aurea* Decne and *Phlomis floccose* D. respectively (table 1), flavonoid contents of the extracts were calculated in terms of quercetin equivalent (the standard curve equation: y= 0.0067x ± 0.0132 r^2 = 0.999), (Figure 4).

The total phenylethanoid glycosides′ content was estimated using Arnow′s reagent and verbascoside as standard where each set of calibration standards absorbance (AU) versus concentration, was fitted to a least squares linear plot, all plots were found to be linear across the assayed range (2.00-8.00 µg/ml, R2 = 0.9964), the calibration curve was used to calculate the percentage of phenylethanoid glycosides′ content (Figure 4). The nanoparticulated extracts exhibited elevated levels (22.85 ± 1.92 &21.40 ± 1.51 mg %) than the crude extracts (18.60 ± 1.25 &18.25 ± 1.46 mg %) of *Phlomis floccose* D. and *Phlomis aurea* Decne respectively (table 1) while the total iridoid glycoside content was performed using Trim and Hill reagent and herbagoside as standard where each set of calibration standards absorbance authentic versus concentration, was fitted to a least squares linear plot, all plots were found to be linear across the assayed range (2.00 - 8.00µg/ml, R2 =0.9964), the calibration curve was used to calculate the percentage of iridoid content (Figure 4). The nanoparticulated extracts exhibited elevated levels (11.03 ± 1.02 & 10.15 ±0.88 mg %) than the crude extracts (9.40 ± 0.95 &8.65 ± 0.72 mg %) of *Phlomis aurea* Decne and *Phlomis floccose* D. respectively (table 1).

Qualitative TLC-DPPH assay of the tested extracts showed that they are active compounds as DPPH scavengers appearing as zones with different R_f values at in the chromatogram, then quantitative estimation of the antioxidant

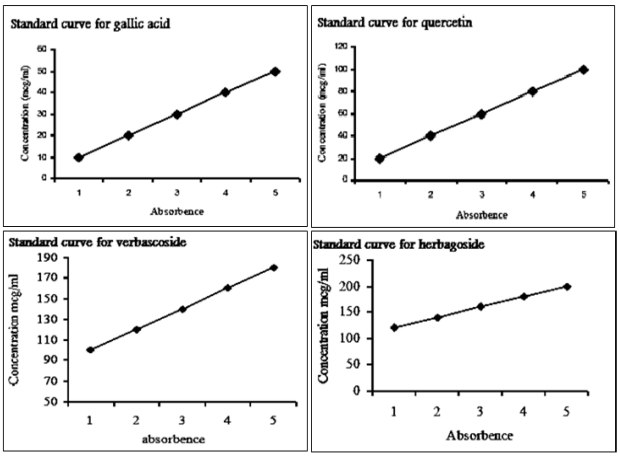

Fig 5: Standard curves for gallic acid, quercetin, verbascoside and herbagoside.

potentials was carried out spectrophotometrically using DPPH method at two dose levels (50 & 100 mg %) revealed that they possesses significant free radical scavenging activity which is proven comparable to the reference synthetic antioxidant butylated hydroxytoluene (BHT) in dose dependent manner, the most significant percent free radical scavenging effect indicated as the least IC_{50} i.e. the higher potencies were recorded for the nanoparticulated extracts (67.83 ± 2.77 & 62.05 ± 3.96 and 95.50 ± 1.84 & 93.05 ± 1.75 µg ml^{-1}) than the crude extracts (55.45 ± 2.52 & 49.24 ±2.65 and 88.44 ± 3.90 & 84.45 ± 1.51 µg ml^{-1}) of *Phlomis aurea* Decne and *Phlomis floccose* D. at dose levels 50 and 100 µg ml^{-1} respectively (Table 2).

Table 1: Total phenolic, flavonoid, phenylethanoid glycosides′ and iridoid contents of crude methanol extracts of *Phlomis aurea* Decne and *Phlomis floccose* D. (FACE & PFCE) and silver nanoparticulated extracts (PAAgNPs & PFAgNPs):

Total Content (mg %)	*Phlomis aurea* Decne		*Phlomis floccose* D.	
	PACE	PAAgNPs	PFCE	PFAgNPs
Total Phenolic	51.73±2.75	64.96 ± 2.90	49.33 ± 2.71	62.40±2.85
Flavonoid	29.50±2.25	32.16 ± 2.05	27.58 ± 2.14	29.43±1.95
Phenylethanoid	18.25 ± 1.46	21.40 ± 1.51	18.60 ± 1.25	22.85 ± 1.92
Iridoid	9.40 ± 0.95	11.03 ± 1.02	8.65 ± 0.72	10.15 ±0.88

*Data represented by means ±standard error of triplicate experiments.

Table 2: Percent DPPH free radical scavenging of different concentrations of crude methanol extracts of *Phlomis aurea* Decne and *Phlomis floccose* D. (FACE & PFCE), silver nanoparticulated extracts (PAAgNPs & PFAgNPs) and the reference standard butylated hydroxytoluene "BHT":

Dose (µg ml^{-1})	*Phlomis aurea* Decne		*Phlomis floccose* D.	
	PACE	PAAgNPs	PFCE	PFAgNPs
50	55.45 ± 2.52	67.83 ± 2.77	49.24 ±2.65	62.05 ± 3.96
100	88.44 ± 3.90	95.50 ± 1.84	84.45 ± 1.51	93.05 ± 1.75
ED_{50}	1.33±0.028	1.10±0.025	1.59±0.030	1.28±0.022

* Data represented by means ±standard deviation of triplicate experiments. *EC_{50} for Butyl hydroxyl toluene (BHT) "Antioxidant standard" =0.054 µgml^{-1}.

In vitro cytotoxicity assays have been employed for screening of the crude and nanoparticulated extracts of *Phlomis aurea* Decne and *Phlomis floccose* D. for their anticancer activities, this was carried out at dose levels of 0.780, 1.560, 3.125, 6.250, 12.5, 25, 50 and 100 µg/ml on different cell lines HEPG-2, HCT-116 and MCF-7 exhibited the enhanced cytotoxic activity of the nanoparticulated extracts compared to the crude ones, this was manifested by their reduced IC_{50} for different used cell lines.

Table 3: Percent inhibition of cell viability of different concentration of crude methanol extracts of *Phlomis aurea* Decne and *Phlomis floccose* D. (FACE & PFCE), silver nanoparticulated extracts (PAAgNPs & PFAgNPs) and their calculated IC_{50} for different cell lines:

***Phlomis aurea* Decne**						
Conc. (µg ml^{-1})	**PACE**			**PAAgNPs**		
	HEP-G2	**HCT-116**	**MCF-7**	**HEP-G2**	**HCT-116**	**MCF-7**
100.00	98.91±3.65	98.90±2.75	62.16±2.55	91.54±3.65	98.46±3.31	69.74±2.80
50.00	95.47±3.51	96.05±3.19	53.91±2.35	84.31±3.20	96.97±3.54	51.27±2.71
25.00	92.83±3.24	92.48±3.05	45.86±2.26	75.22±3.03	91.81±3.70	38.52±2.09
12.500	87.42±3.18	87.91±3.28	37.68±2.15	63.07±2.94	84.54±2.85	27.68±1.89
6.250	73.06±2.97	71.83±3.00	23.33±1.90	42.57±2.40	68.17±2.65	20.41±1.80
3.125	47.52±1.75	58.62±2.86	12.54±1.05	31.86±2.15	52.83±1.99	11.93±1.17
1.560	34.18±2.05	45.78±2.69	8.97±0.95	15.81±1.96	36.94±2.07	8.08±0.96
0.780	20.97±1.35	31.89±2.10	5.41±0.86	8.65±1.16	23.72±1.84	4.73±0.84
0.00	0.00	0.00	0.00	0.00	0.00	0.00
IC_{50}	8.92±0.84	7.55±0.75	49.75±1.95	11.85±1.03	5.50±0.13	38.85±2.05
***Phlomis floccosa* D.**						
Conc. (µg ml^{-1})	**PFCE**			**PFAgNPs**		
	HEP-G2	**HCT-116**	**MCF-7**	**HEP-G2**	**HCT-116**	**MCF-7**
100.00	96.50±3.51	98.25±2.95	79.95±2.74	88.60±3.11	95.60±3.46	66.50±2.71
50.00	93.45±3.18	95.92±3.09	70.30±2.69	81.40±2.96	93.25±3.51	55.10±2.40
25.00	89.51±3.13	89.90±3.15	63.55±2.37	72.15±2.58	88.10±3.35	36.90±1.96
12.500	85.25±2.90	86.45±3.01	44.65±2.10	61.40±2.42	81.59±3.28	24.51±1.85
6.250	72.55±2.73	69.08±2.81	22.10±1.89	40.35±2.29	66.65±2.89	19.15±1.52
3.125	45.70±2.29	56.82±2.49	10.65±1.13	29.62±2.15	50.94±2.75	9.50±1.03
1.560	33.05±2.09	43.25±2.18	7.10±1.06	13.90±1.69	34.75±2.15	6.85±0.79
0.780	18.22±1.63	28.06±1.94	4.65±0.75	7.90±1.08	21.55±1.99	3.97±0.25
0.00	0.00	0.00	0.00	0.00	0.00	0.00
IC_{50}	11.25±1.85	9.50±1.36	40.25±2.55	13.80±1.05	8.85±0.90	33.85±2.10

*Values are results of three experiments presented as means ± standard errors. *$IC_{50\ Cisplatin}$ for HEP-G2 is 0.87, HCT-116 is 0.71 and MCF-7 is 0.62 µg ml^{-1}.

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