ISSN: 2348-1900
Plant Science Today
http://www.plantsciencetoday.online

OPEN ACCESS

Research Paper

SPECIAL ISSUE on Current Trends in Plant Science Research

Antioxidative properties of isolated saponins of *Verbesina encelioides* (Cav.) Benth. & Hook. f. ex Gray and SEM studies of synthesized green nanoparticles for acne management

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Article history
Received: 27 November 2019
Accepted: 28 December 2019
Published: 31 December 2019

Publisher
Horizon e-Publishing Group

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Abstract

Acne is one of the most common and chronic skin problems in most adolescents and young adults due to several internal and external factors. The present study emphasizes on screening of high antioxidant potential of wild sunflower for acne therapy, as it also plays a major role in the patho-physiology of acne. The anti-oxidant potential of extracted plant compounds was carried out by using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). In recent years, scientists have been involved in the application of green nanoparticle synthesis. Further synthesized green nanoparticles were checked for antiacne potential. Isolated saponins and their synthesized nanoparticle would play an important role to control the acne.

Keywords: 1, 1-diphenyl-2-picryl hydrazyl (DPPH); ferric reducing antioxidant power (FRAP); green synthesis; anti-acne herbal formulations

Citation: Verma V, Chaudhary M, Srivastava N. Antioxidative properties of isolated saponins of *Verbesina encelioides* (Cav.) Benth. & Hook. f. ex Gray and SEM studies of synthesized green nanoparticles for acne management. Plant Science Today 2019;6(sp1):575-582. https://doi.org/10.14719/pst.2019.6.sp1.675

Indexing: Plant Science Today is covered by Scopus, Web of Science, BIOSIS Previews, ESCI, CAS, AGRIS, UGC-CARE, CABI, Google Scholar, etc. Full list at http://www.plantsciencetoday.online

Introduction

Since ancient times, medicinal plants have been used for the treatment of human diseases, as they have therapeutic phytochemicals; are more natural and healthy than artificial drugs (1). Plants has abundant amount of secondary metabolites, like phenols, saponins, tannins, alkaloids and flavonoids. There are several plants abundant with antioxidant rich compounds that protect the human body from free radicals and prevent chronic disease. Research is being done in this field, regarding utilization of these natural antioxidants in various pharmaceutical sectors (2).

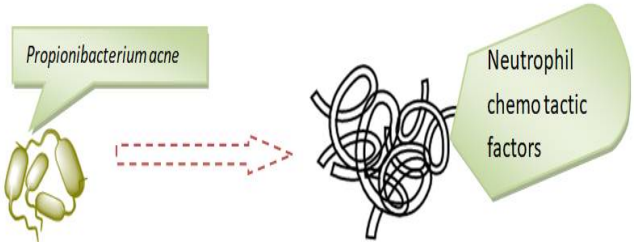
Verbesina is a genus, native to South-Western United States and Mexican plateau, primarily it occurred in the arid grass and

scrublands. *Verbesina encelioides* (Cav.) Benth. & Hook. f. ex Gray, belongs to Asteraceae family with bright yellow flowers, it is a noxious weed that has extended its portfolio to various American countries, South Africa, Australia and India. This plant species has ornamental and economic value also it has tendency to withstand in adverse climatic conditions like drought and heat (3). For indigenous people, weeds are known to be an important source of medicine (4). Phytochemical investigation of *V. enceloides* revealed the presence of flavonol glycosides, primary metabolites (5), triterpenoids (β-sitosterol-D-(+) glucoside, pseudotaraxasteryl acetate, β-sitosterol and hentriacontanol, pseudotaraxastenone) (6) and three rare flavonol glycosides (quercetin-3-xyloside-7-glucoside, quercetin-3-galactoside and quercetin-3-galactoside-7- glucoside) (7). Pharmacological investigation of *V. enceloides* revealed activities like antimicrobial, antiviral, anti-tumor, hypoglycaemic and anti-implantation activity (4, 8). In folk medicines, traditionally *V. enceloides* finds uses for treatment of various disorders viz., cancer, snake-bite, digestive disorder, skin conditions and hemorrhoids. An active antihyperglycaemic agent, Galegine has been used ethnomedicinally to treat diabetes (10). Various studies have been done on explore whole plant to reveal their antifungal, antiviral, anti-implantation, hypoglycaemic antibacterial, antitumor activities (8, 11) antidiabetic, antioxidant, anticancer and antiobesity (12). Saponins are bioactive compounds distributed in plant kingdom which contain either a steroid or a triterpenoid (aglycone) attached to the sugar side chain (13). They act as defensive safeguard for the plant to encounter the pathogens (14). Because of their unique surfactant properties, they are used in cosmetic products also they have an additional emollient effect (moistening) which can be used in various ointment preparations (15).

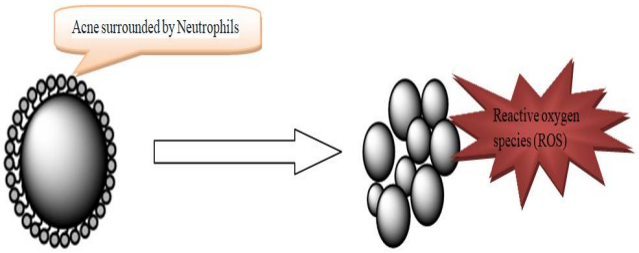
Acne vulgaris is a common skin condition caused due to hormonal imbalance, microbial infection and low immune factors. This affects all age groups, *i.e.* teenagers (85%), 25-34 years (8%) and 35-44 years (3%), and is basically characterized by the presence of bacteria, *Staphylococcus aureus* and *Propionibacterium acnes* in the follicular canal. This is not a detrimental disease, an individual is affected physiologically and emotionally (16). People suffering from acne have low level of antioxidants in their blood, as they are used up constantly, resulting in increased demand of free radicals by body for defense mechanism (Fig. 1).

The potential bioactive which is introduced to defend acne must have ability of inhibiting growth, so molecule used possess high antioxidant potential as well as good antibacterial activity against *P. acnes* (17).

Reactive oxygen species (ROS) act as stimulus for initiation of various biological responses resulting in reduction of immune system. Antioxidants are used as free radical scavengers for removal of ROS to lessen cell injure that occurs during acne inflammation (18). Antioxidant compounds have ability to arrest disable and refurbish the harm caused by free radicals (19).



Step 1. *P. acne* envoke inflammation of NCF (Neutrophil chemotactic factor)



Step 2. Inflammatory mediators such as ROS (hydroxyl (•OH)), super oxide (O2•-) and reactive nitrogen (RNS) species are continuously released and when neutrophils are attracted to acne lesions)

Fig. 1. Diagrammatic representation to understand relationship between acne and ROS.

Nanotechnology is the development of nano-scale materials for the purposes of innovation (20). Nano-medicine aims to exploit the new properties for diagnostic and therapeutic uses of engineered nanomaterials (21, 22). In recent years, it became clear that nano-material tissue interactions at the cellular and systemic levels were required to optimize therapeutic applications and reduce potential negative effects (23). Therefore, Nanotechnology relates not only to nanoscale, but manufacture, develop and design materials with precision and specificity (24). For a range of nanomaterials, skin is foremost point of contact–from topical procedures, articles of clothing, domestically produced utensils, athletic goods and industrial products. Newer approaches for medical diagnosis, follow-up and treatment also refer to nanomedicine in dermatology (25).

For synthesizing nano-particles, plants serve a better platform, as they are free from toxic substances and provide natural cappers (26). Silver nanoparticles (AgNPs) have distinctive features in catalysis, chemical sensing, bio-sensing; photonics as well as pharmaceutical products (27). For biological activities, silver nanoparticles also have great potential in pharmaceutical industry (28).

2. Materials and Methods

2.1 Plant materials

The flowers of *Vebesina encelioides* were collected during the month of the July from the campus area of Banasthali Vidyapith, Rajasthan. The flower was authenticated in the Department of Bioscience and Biotechnology (Botany), Banasthali Vidyapith.

2.2 Chemicals and Microbial culture

All the chemicals were procured from Sigma Chemical Company Pvt. Ltd., St. Louis, MO, USA. Acetonitrile and methanol (HPLC Grade), Sterile Disc (Hi-media) and standard microbial culture of *Propionibacterium acne* (MTCC 1951) were procured from IMTECH, Chandigarh, India.

2.3 Extraction of the crude saponins

Flowers of *Vebesina encelioides* were collected washed and shade dried and milled into dry powder. 50% aqueous methanol was used as an extraction solvent. The extraction was done with 1 g of *V. encelioides* powder was extracted for 72 hrs at room temperature with 10 ml of 50% aqueous methanol on rocker shaker. The filtrate was collected and separated by two-phase extraction with water-saturated butanol. n-Butanol layer after two phase separation was collected separately and aqueous layer was retain and concentrated for further evaluation.

2.4 Determination of Total Saponin content (TSC)

Total saponin content (TSC) determination was carried out (29, 30). In a conical flask, 5 g of powder was taken and 20% ethanol was added. The mixture was heated for 4 hrs with constant stirring in water bath at 55 °C. The left over marc (solid residue) was extracted again according to the previous step with another 20% ethanol and extract were combined and evaporated in water bath at 90 °C. After evaporation, the extract was poured in a separatory funnel and diethyl ether was added followed by vigorous agitation to develop two layers, the aqueous layer formed was retained while the ether layer was discarded. The extract was kept overnight to evaporate ether. n-butanol was added into funnel. Two layers developed, butanol layer was discarded and aqueous layer was kept overnight for concentrate. The extract was transferred into a petridish and was again dried in an oven to a constant weight. The saponin percentage was calculated using equation:

$$\% \text{ Saponins} = \frac{\text{Weight of the petriplate with extract - Weight of the petriplate}}{\text{Weight of the sample}} \times 100$$

2.5 Purification and Characterization of isolated saponins from the extract

2.5.1 Silica gel column chromatography and TLC analysis

The extract was then separated using silica gel column chromatography, dried extract was dissolved in 1 ml of petroleum and was loaded onto column and fractions were collected from elution solvents in the spectrum of polar to non-polar for a given period of time.

The extract was loaded over TLC sheet using capillary, ethyl acetate and methanol (3:1) was used as mobile phase and visualization was done using 5% vallinin-sulphuric acid spray followed by heating plates at 100 °C in oven.

2.5.2 Identification and characterization of saponin from plant extract

Fourier Transform Infrared (FTIR) spectroscopy (Bruker Alpha-T spectrometer) was done for the identification of different functional groups present in the extract (31) and for separation and identification of saponin high-performance liquid chromatography (HPLC) (Shimadzu LC-10A) was performed using column C-18 by acetonitrile and methanol as mobile phase (gradient elution) with UV–Vis detector at wavelength 254 nm at optimum flow rate 1 ml/min and injection volume 20µl (32).

2.6 Free radical scavenging activity (antioxidant Assay)

The free radical scavenging activity of the plant extract was calculated using the DPPH activity and FRAP assay.

2.6.1 DPPH radical scavenging assay

The DPPH radical scavenging assay of plant extract was evaluated by a published method (33) with slight modifications. Antioxidants on reaction with DPPH get converted into 1, 1-diphenyl-2-picrylic hydrazine which is visualized by change in color from violet to yellow. Different concentration (0.1-0.5 mg/ml) of the extract was added and methanol was added to make volume up to 2 ml. To each tube 1 ml of freshly prepared DPPH solution was mixed in plant extract. Absorbance was recorded at 517 nm using UV-VIS Spectrophotometer UV-2450 (Shimadzu) and antioxidant activity of extract was calculated as:

$$IC_{50} = \frac{\text{Absorbance of control - Absorbance of control}}{\text{Absorbance of control}} \times 100$$

DPPH scavenging activity is expressed as milligram of Ascorbic acid equivalent per gram of sample. Graphically the value is calculated by plotting the absorbance (% inhibition of DPPH radicals) against the log concentration of DPPH.

2.6.2 FRAP Assay

The assay was carried out according to a standard procedure (34). The basic reaction involved is reduction of Fe^{3+} TPTZ complex (colorless) to Fe^{2+} tripyridyltriazine (deep violet). Different concentration of plant extract was mixed with FRAP reagent (Acetate buffer (10):TPTZ(1): $FeCl_3.6H_2O$(1)) resulting in formation of deep violet complex which was recorded at 593 nm after incubation of 30 min at 37 °C. The FRAP values

expressed as mg of ascorbic acid equivalent per gram of sample.

3. Green synthesis of silver Nanoparticles

Synthesis of green silver nanoparticles was done (35). In a beaker, 0.1M aqueous silver solution was prepared in de-ionized water and 2 ml of plant extract was added. It was kept over magnetic stirrer in dark for the bio-reduction process.

3.1 UV–Vis absorbance spectroscopy analysis

2ml of colloidal solution was taken from 0 to 24 hrs to analyze the reaction between silver ions and saponins which was recorded at a scanning range of 200–600 nm using a UV–Vis spectrophotometer (36).

3.2 Fourier Transforms Infrared Spectroscopy (FTIR)

The colloidal solution of synthesized nanoparticles was then analyzed for presence of various functional groups in nanoparticles with the FTIR.

3.3. Scanning Electron Microscopy (SEM) of synthesized silver nanoparticles

The morphology and size of the synthesized green nanoparticles were further characterized by high-resolution SEM (TESCAN). The nanoparticles were palladium-coated (37).

3.4 *In Vitro* anti-acne activity of synthesized nanoparticles

Antimicrobial assay of saponins conjugated-silver nanoparticles was done by disc and agar well diffusion method at varying concentrations. The overnight grown bacterial culture of *Propionibacterium acne* was spread over nutrient agar. Sterile disc was impregnated with 50 µl of the test sample and 20 µl of antibiotic. The disc was air dried before impregnating on to plate. For agar well method, wells were scooped out and 50 µl of sample and 20 µl of the antibiotic was pipette into well. Plates were then incubated at 37 °C in the incubator. Next day the zones of inhibition around the discs were measured in terms of millimeters (mm) (38).

3.5 Statistical analysis

For analysis of antioxidant capacity of the extracts, the assay was performed in triplicate, and the results were expressed by means ± SD (standard deviation).

4. Results and Discussion

4.1 Total saponin content (TSC) determination

Saponin is distributed in various parts of plants and participates in defense mechanisms against pathogens. Therefore; serves as potential candidates for preparations of various medications. In the present study, TSC content in flower of *V. enceloides* was calculated quantitatively and percentage of saponin is 7.68% per 5 g of dry weight. Previous study reported that, total saponin content in *Mandshurica aralia* was 9.41 to 10.4 percent (39). In wild *Paris polyphylla*, the total saponins content content in the rhizome is 2.021 and in the root the content is 0.263% (40). Similar finding were reported for methanol extract of *maca* leaves, total saponin content recorded was 6.09 mg / 100 g and in *maca* roots was 5.10 mg / 100 g (41).

4.2 Chromatographic characterization of crude saponin

The saponin isolated from the plant was then chosen for thin layer chromatography studies which revealed various spots on sheet but one spot with R_f 0.59 appeared at same height of standard (Fig. 2).

Fig. 2. TLC of the extract with reference to standard.

The TLC analysis of *Moringa oleifera* revealed 13 components of saponin which was recorded at 254 nm with R_f values in between 0.01-0.87 (42).

Extract was subjected for quick analysis by HPLC where the separation of compounds was analyzed by identification of various peaks present on chromatogram. In HPLC analysis, the extract showed good separation of peaks in standard solution (Rebaudioside A) identified on chromatogram with retention time (RT) 6.75 min (Fig. 3a) while the isolated saponins showed the peak on chromatogram with retention time (RT) 6.8 min (Fig. 3b) which confirmed the presence of saponins as chromatograph obtained are quite similar. Previous reports, found that extracts of *Bupleurum falcatum* revealed presence of

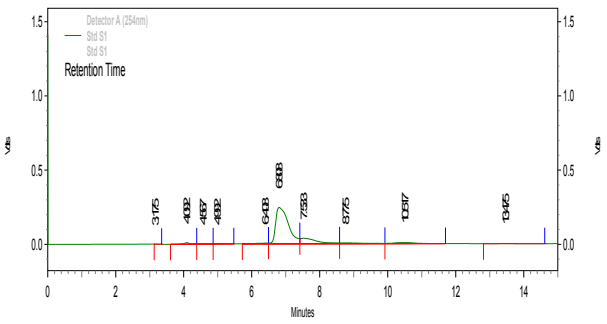


Fig. 3. (a) HPLC Graph of Saponin standard Rebaudioside A.

saikosaponin a, c and d complex through reversed-phase (ODS C18) column (43).

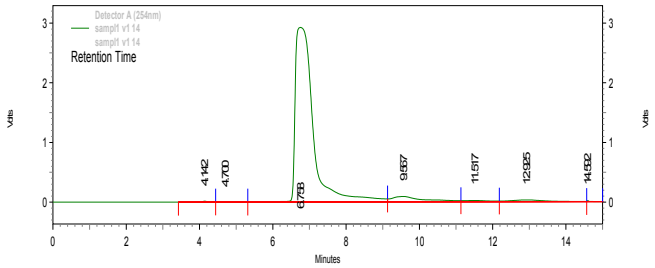


Fig. 3. (b) HPLC Graph for isolated saponin from extract

4.2 Antioxidant activity

4.2.1 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical-scavenging activity of the aqueous methanol extract of *V. enceloides* was investigated to determine its antioxidant property. DPPH radical scavenging activity of the aqueous methanol extracts increased with increase in concentration. The findings are expressed as percentage of inhibition against concentration in Fig. 4. IC50 value observed from the graph range between 200-300 mg/ml of absorbic acid.

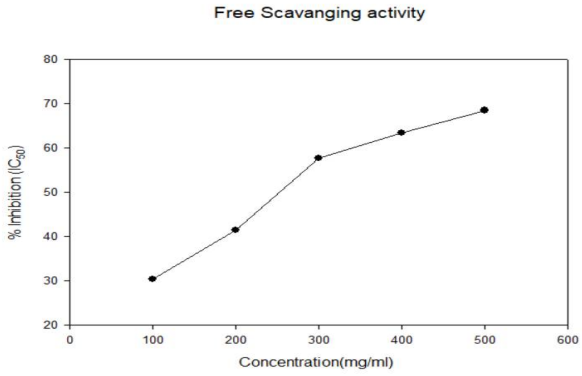


Fig. 4. Percentage free radical scavenging and extract concentration curve of *V. enceloides.*

Previous reports showed that saponin derivatives of *Hedera helix* L. (Araliaceae) plant extract showed significant antioxidant capacity and can be used as a natural antioxidant origin (44). It was reported that saponin isolated from *Leontice smirnowii* Trautv (Berberidaceae) showed high antioxidant activity (45).

4.2.2 Ferric reducing ability of plasma assay (FRAP)

FRAP assay provide an estimation of antioxidant present in the aqueous methanol extract of flower. This assay is based on the ability of extract to reduce Fe^{3+} to Fe^{2+} pair. The aqueous methanol extract exhibited significant FRAP activity. As the absorbance of the plant extract is high, reducing power would be higher. Present study showed, reducing power of the extract increased with the increase in concentration of the extract (Fig. 5).

5. Green synthesis of saponin conjugated AgNPs

During synthesis of nanoparticles specific color change is observed (46). In the present study, the formation of AgNPs was observed by monitoring the change of color. The synthesis of the AgNPs was recorded by a color change from colorless to brownish (Fig. 6).

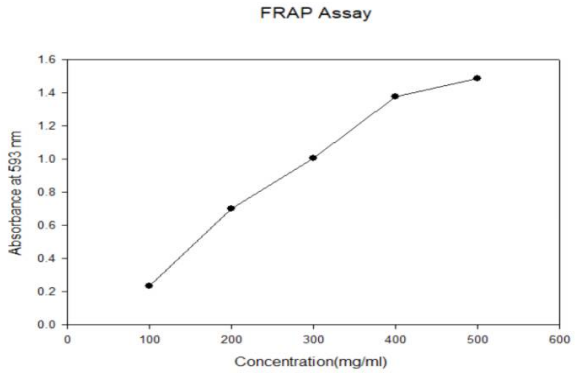


Fig. 5. FRAP assay of the aqueous methanol plant extract

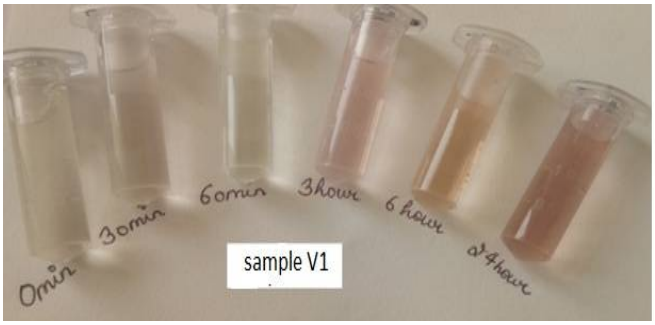


Fig. 6. Synthesis of the silver nanoparticles of crude saponin from sample from 0-24 hrs.

5.1 Characterization of nanoparticles

The synthesized nanoparticles were analyzed spectrophotometrically that measured bio-reduction of aqueous silver ions and also recorded Surface Plasmon band which is part of optical absorbance spectra of silver nanoparticles (47). The saponin conjugated AgNPs showed strong absorption peaks at 410 nm (Fig. 7).

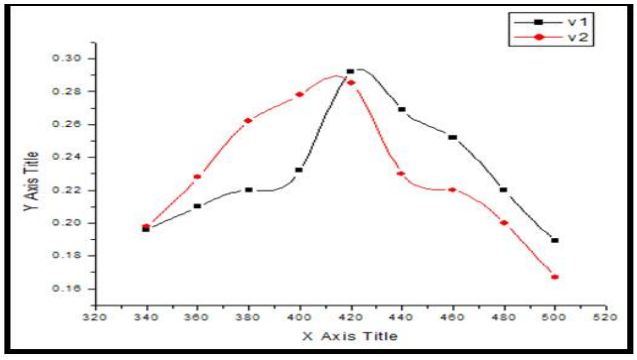


Fig. 7. UV-Visible absorption spectra of silver nanoparticles of plant extract (v1= control and v2=extract).

After synthesis of silver nanoparticles of our green extract, further characterization was done by FTIR and SEM. Fourier Transform Infrared spectroscopy analyze different functional groups present in synthesized AgNPs also the

spectrum analysis showed stabilization (capping) of nanoparticle and various distinct peaks at 2924.95 cm^{-1}, 1741.85 cm^{-1}, 1546.81 cm^{-1}, 1515.29 cm^{-1} and 1367.81 cm^{-1} were obtained and corresponds to methylene, aldehydes, carboxylic acid, aromatic ring, amines and amides and polysaccharide respectively (Fig. 8).

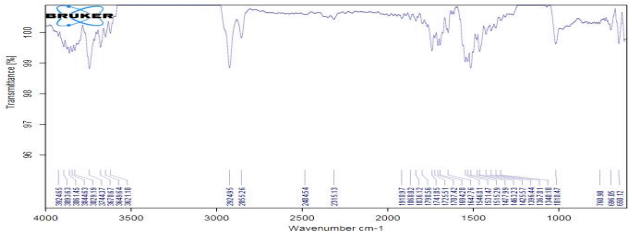


Fig. 8. FT-IR analysis of AgNPs obtained from isolated saponins.

Scanning electron micrograph (Fig. 9) of the AgNPs obtained reveals uniformly distributed round to spherical shaped saponins conjugated-silver nanoparticles on the surface of the cells with diameters ranging between 40 to 43 nm in size are depicted.

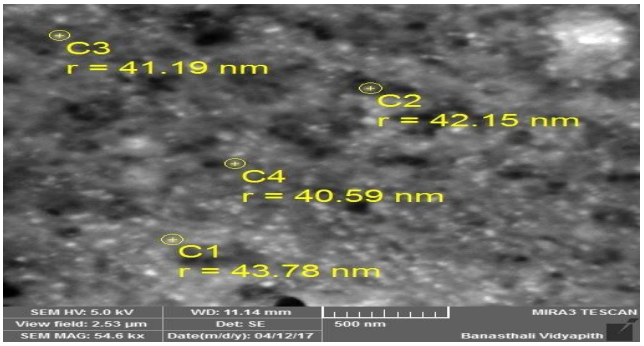


Fig. 9. SEM image of the synthesized nanoparticles

These results have proved the reducing ability of *V. enceloides.* Zeta studies revealed the average size of 94.45 nm with stability (potential) of -26.7 mV (Fig. 10).

In a recent study, AgNPs synthesized from butanolic extract of *the Chenopodium album* revealed an average size of 30 to 40 nm in diameter (47). Similar finding were reported from the aqueous extract of *Eclipta prostrata* where the normal size of nanoparticles recorded was 35–60 nm in diameter. SEM analysis of *Euphorbia hirta* leave extract showed spherical shaped nanoparticles with size range of 40–50 nm diameter (48).

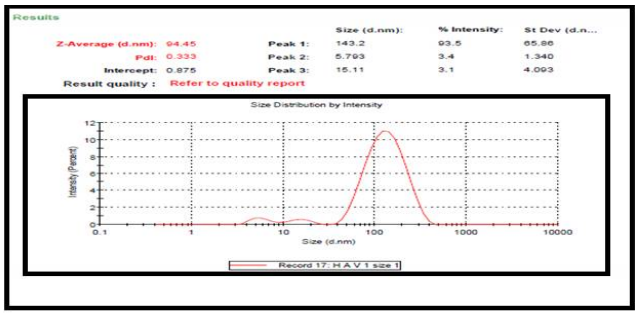


Fig. 10 (a)

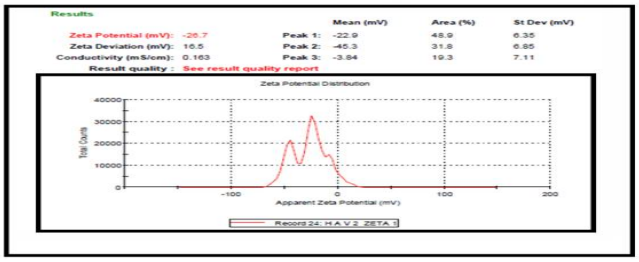
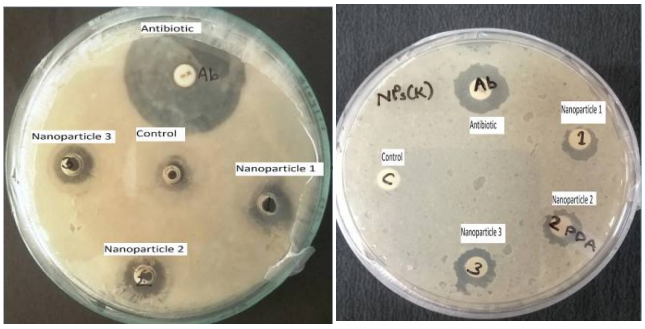


Fig. 10 (b)

Fig. 10. Size and potential stability of saponin-AgNPS by zeta analyser (a) Zeta size (b) Zeta potential of the NPs from aqueous methanol extract of *V. menceloides*.

5.2 Antimicrobial activity of AgNPs synthesized from saponin isolated from aqueous methanolic extract of *V. enceloides*

Antimicrobial activity of the synthesized AgNPs was studied against *Propionibacterium acne.* Saponins conjugated silver-nanoparticles shows potential against acne-causing bacteria in both the method. Zones of inhibition measuring 11 nm, 12 mm, 12 nm respectively was observed in the disc diffusion method while agar well diffusion method zones of inhibition recorded were 10 nm, 11 mm and 12 nm (Fig. 11).



(a) (b)

Fig. 11. *In vitro* antimicrobial activity of the synthesised saponins conjugated nanoparticles at varying concentration (a) agar well diffusion(b) Disc diffusion method (Nanoparticles 1, 2, and 3 (same concentration), control: silver nitrate, antibiotic: kanamycin).

The synthesized nanoparticles have performed potential role against acne-causing bacteria. Similar results were obtained from synthesized saponins-AgNPs from aqueous extract of *T. decandra* that exhibited excellent activity against *P. vulgaris, S. aureus, S. faecalis, Y. enterocolitica* and *E. coli* with zones of inhibition measuring 7.8 mm and 20.3 mm (49).

Conclusion

The present study reveals the antioxidant potential of aqueous methanol extract of flower of *V. enceloides* is due to presence of saponin content in the significant amount and is also responsible for reduction process of Ag^{+} to Ag^{0} during synthesized silver nanoparticles (AgNPs).

Hence, it is possible to conclude that plant extract have considerable free radical scavenging activity and can be used as strong natural

antioxidant agents and synthesized AgNPs showed significant antibacterial activity against *P. acne*. SEM studies of green silver nanoparticles have been depicted 40-43 nm size nanoparticles.

Our results suggest that aqueous methanol extract flower of *V. encelioides* has promising antioxidant potential and anti acne activity. Therefore, it can be further used to develop various medicines, cosmetics and various ointments.

Conflict of interest

The author declard no conflict of interest.

Authors' contributions

VV has performed the experiment, done data analysis and prepared the initial manuscript under the supervision of NS; NS has designed the whole concept of the experiment, analyzed data, edited and finalized the whole manuscript; MC has helped in the experiment along with the compilation of the whole data.

Acknowledgements

The authors are grateful to Prof. Aditya Shastri, Vice Chancellor, Banasthali Vidyapith for providing all necessary support. We acknowledge the Bioinformatics Center, Banasthali Vidyapith supported by DBT for providing computation support and DST for providing networking and equipment support through the FIST and CURIE programs at the Department of Bioscience and Biotechnology. CESME, Banasthali Vidyapith, supported by MHRD, Government of India under the PMMMNMTT is acknowledged for organizing the symposium.

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