RESEARCH ARTICLE

PHARMACOLOGICAL AND NANOBIOTECHNOLOGICAL ASPECTS OF GYMNEMA SYLVESTRE

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Abstract

Gymnemasylvestre commonly known Gurmar or sugar destroyer, belonging to the family Asclepiadaceae, is a slow-growth, woody climber distributed throughout India, traditionally used in Ayurvedic system of medicine for the treatment of several diseases such diabetes, jaundice, urinary complaints, chronic cough, stomach problems, breathing troubles, malaria and snakebites. The present work was planned to investigate phytochemical profiling and green synthesis of silver nanoparticles using Gymnemasylvestre. The phytochemical assessment of stem and leaf extracts of Gymnemasylvestre revealed the presence of important phytocompounds such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, proteins, saponins, steroids, tannins and terpenoids. Saponin was found in all the sample extract. Aqueous stem extract of Gymnemasylvestre was utilized for the synthesis of silver nanoparticles. UV-Vis spectrophotometric study exhibited absorbance peak at 419 nm that measures the reduction of silver nitrate to silver nanoparticles based on optical properties called surface plasmon resonance. FT-IR study of silver nanoparticles showed the presence of specific molecular functional groups such as alkenes, amines, amides, alcohols, phenols, aromatic and halo compounds that acts as capping and stabilizing agents for the synthesis of silver nanoparticles. XRD pattern analysis suggested that the crystalline nature of silver ions and crystalline size of silver nanoparticles of 39.5 nm by Scherrer’s formula. The presence of this phytocconstituent suggested vital medicinal features of the plant and their importance in synthesizing and stabilizing silver nanoparticles.

Introduction:

Medicinal plants are a major source for discovery and development of new drugs or lead compounds because of their diversified specific chemical structure and unique mechanisms of action that have a wide variety of biological targets. From the beginning of human life, herbal medications are basic need or vital resource for the health management system. According to World Health Organization (WHO), 80% of the world population entirely depends on herbal therapeutic ingredients for basic health needs (Winter and Tang, 2012; Mohanraj et al., 2018). Traditional medicines derived from herbal plants play a significant role in the management of many ailments like diabetes, arthritis, cardiovascular disorders, asthma etc. A scientific investigation of traditional herbal medications for the treatment of many diseases may provide valuable knowledge for the development of alternative drugs and healing approaches (Kumar et al., 2015).

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Nowadays, in developing countries the herbal medications are becoming prominent due to better result, easy availability to common individuals, low side effects, quality, safe use, and wide pharmacological applications as compared to marketed drugs and more potential in health problem treatment, thus the demands for herbal medications have drastically increased (Vikram et al., 2014). Many plant species contain many valuable bioactive compounds which are isolated and administrated directly as drugs, lead compounds and have pharmacological importance. Traditional approaches derived from medicinal plants provide a natural key to unlock many health-related complications (Tiwari et al., 2014).

India has enriched with indigenous medicinal flora that has been used in Indian traditional medications from the prehistoric era for the treatment of several human ailments. Herbal plants are the major source of drugs in the Indian medicine system and also in other prehistoric systems in the world (Jayachitra and Muniyandi, 2016). Phytoconstituents of herbal plants possess active compounds widely used in drug discovery (Subramaniyan and Srinivasan, 2014).

The present work is focused on traditionally utilized a valuable medicinal plant Gymnemasylvestre with an overview of its phytochemical investigation and green synthesis of silver nanoparticles. Gymnemasylvestre a potent antidiabetic plant, used in homoeopathy, folk and ayurvedic system of medicine, which belongs to family Asclepiadaceae, a vulnerable species, commonly known as Periploca of the woods in English, Madhunashini in Sanskrit and Gurmar in Hindi which means “destroyer of sugar” (Mitra et al., 1995; Kumar, 2015). Gymnemasylvestre is also being widely used for the treatment of eye complaints, inflammations, asthma and snake bite by tribal population (Anonymous, 1956; Selvanayagam et al., 1995).

National Botanical Research Institute, Lucknow, India conducted a floristic study in 7 districts of Chhattisgarh state and enlisted Gymnemasylvestre R. Br. among 44 other species in the list of Endangered Taxa of Chhattisgarh state, India (CGMPB, Raipur, Chhattisgarh, 2017).

**Taxonomy of the plant**

**Taxonomical classification of Gymnemasylvestre**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subkingdom</td>
<td>Tracheobionta</td>
</tr>
<tr>
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<td>Spermatophyta</td>
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<td>Asclepiadaceae</td>
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<tr>
<td>Genus</td>
<td>Gymnema</td>
</tr>
<tr>
<td>Species</td>
<td>sylvestre (Duke et al., 1997; Kritikar and Basu, 1999)</td>
</tr>
</tbody>
</table>

**Vernacular name of Gymnemasylvestre**

<table>
<thead>
<tr>
<th>Language</th>
<th>Vernacular Name</th>
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</thead>
<tbody>
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<td>Arabic</td>
<td>Barkista</td>
</tr>
<tr>
<td>Bengali</td>
<td>Meraasingi</td>
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<tr>
<td>English</td>
<td>Periploca of the woods</td>
</tr>
<tr>
<td>Hindi</td>
<td>Gurmar</td>
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<td>Malayalam</td>
<td>Cakkarakkoli</td>
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<td>Telugu</td>
<td>Podapatra</td>
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<tr>
<td>Tibetan</td>
<td>Sasinga</td>
</tr>
<tr>
<td>Urdu</td>
<td>Gurmar (Jayachitra and Muniyandi, 2015)</td>
</tr>
</tbody>
</table>
Gymnemasylvestre is a perennial, slow-growing, medicinal woody climber, commonly found in a tropical and subtropical humid environment, belonging to family Asclepiadaceae distributed throughout India (Manohar et al., 2009). The plant is largely found in a tropical forest of Central and Southern part of India and Malaysia, Sri Lanka, Tropical Africa and China (Singh et al., 2008). The leaves are opposite, elliptic or ovate (1.25-2.0 inch x 0.5-1.25 inch). Flowers of Gymnemasylvestresmall in length, the corolla is pale yellow, valvate, campanulate, corona single, with 5 fleshy scales, calyx-lobes are long, obtuse, ovate and pubescent, anther is connective produced into a membrane tip, pollinia 2, erect, carpels 2, unilocular, locules many ovulated, follicle long and fusiform (Tiwari et al., 2014).

The Genus Gymnema includes 40 species distributed from Western Africa to Australia; some important species of Gymnema are G. balsamicum, G. montanum, G. acuminatum (Roxb.), G. latifolium, G. aurantiacum, G. sylvestre R. Br., G. indorum, G. spartum, G. yunnanse. These species of Gymnemagenerally found in Tropical Africa, Vietnam, Sri Lanka, Malaysia, India, Australia and Deccan Peninsula parts of Northern and Western India (Subramaniyan and Srinivasan, 2014; Kumar et al., 2015).

In present scenario the field of nanotechnology is the most dynamic section of research in material sciences and engineering for the synthesis of nanoparticles and that field is emerging significantly all over the world. Nanoparticles expresses completely novel and improved properties into particular features such as shape, size (1 to 100 nm) and structure.

In recent years, green nanotechnology attracted many researchers from various field like physics, chemistry, material science, engineering, medicine and biotechnology. Nanotechnology involves in the process of production, manipulation and also the size of the material ranging from less than a micron to that of an individual atom. The physical, chemical and biological process is the wide variety of method used for the synthesis of nanoparticle (Sastry et al., 2003). Bio-nanotechnology is an emerging branch of science that combines physical and chemical approaches with biological principles to produce nano-sized elements with specific functions.

Silver nanoparticles have an important role in the field of nanotechnology because of their incomparable biological, chemical and physical properties. In recent year, various reliable and eco-accommodating efforts and technology were made to synthesis green methods for synthesis of nanoparticles to avoid hazardous by-products for a reduced level of pollution. Gardea-Torresdey et al., in 2003 first time utilized plant source Alfalfa sprouts for the formation of metallic nanoparticles and first time described plant material based green approach of synthesis of silver nanoparticles (Rafique et al., 2017). Silver nanoparticles have an extensive superstructure which responsible for the vital biochemical activity, catalytic properties and atomic nature that is compared with large elements having the same chemical compositions (Xu et al., 2006).
Materials And Methods:-

Chemical reagents
For present work, analytical grade biochemicals were used and purchased from HI-MEDIA Pvt. Ltd., Mumbai.

Collection of plant materials
Based on the ethnopharmacological records, an important Indian traditional medicinal plant Gymnemasylvestre was selected for the study. This plant was collected from Chhattisgarh State Medicinal Plant Board Raipur, Chhattisgarh during February 2020. Department of Botany, Government V.Y.T. PG. Autonomous College, Durg, Chhattisgarh, identified the plant material. The plant samples leaf and stem of Gymnemasylvestre were rinsed thrice with distilled water followed by double distilled water to remove the dust particles and other contaminants then after that shade dried at 25-30 °C for two weeks followed by the grinding process and the fine powder was kept in the well labelled air-tight glass bottles in darkness at -20°C until further use.

Plant extract preparation
The powdered plant materials from leaf and stem (150 gm each) were extracted successively with six solvents like petroleum ether, benzene, chloroform, methanol and distilled water (aqueous) using Soxhlet apparatus at 60 to 85 °C for 6 to 10 hours to extract the polar and non-polar compounds (Elgorashi and Staden, 2004). Each sample extract was filtered with the help of Whatman filter paper (No.1) and then kept in sterile air-tight glass bottles for further study.

Qualitative phytochemical analysis
Qualitative phytochemical analysis was carried out for the detection of important phytoconstituents like alkaloid, cardiac glycosides, flavonoids, saponins, steroids, tannins, terpenoids, protein, phenol and carbohydrates in the plant sample extracts using the following standard protocols with some modifications (Harborne, 1998; Trease, 2002).

Test for alkaloids
0.5 ml extract, 3 ml of methanol, 300 µl of acetic acid were mixed and then few drops of ammonium hydroxide solution was added. Formation of precipitate showed the presence of alkaloids in the sample extract.

Test for cardiac glycosides
0.5 ml of extract, 0.2 ml glacial acetic acid and dropwise 3.5% ferric chloride were added then layered with 1 ml of conc. sulfuric acid. The formation of a reddish-brown ring at the interface indicated the presence of cardiac glycosides in the sample extract.

Test for flavonoids
0.5 ml plant extract, 5 ml distilled water were mixed in the test tube then it was filtered. 5ml of dilute ammonium solution and then conc. sulfuric acid was added. The formation of yellow colour indicated the presence of flavonoids in the sample extract.

Test for saponin
2.5 ml of extract, 5 ml of distilled water were added and then solution was vigorously shaken and the formation of bubbles or stable persistence of foam indicated the presence of saponins in sample extract.

Test for steroids
0.5 ml of extract, 3 ml of chloroform was added and then the solution was filtered then 2 ml of conc. sulfuric acid was added to the filtrate. The formation of a reddish-brown ring at the interface indicated the presence of steroids in the sample extract.

Test for tannins
0.5 ml of extract, 5 ml of distilled water were added than 1% ferric chloride was added. The appearance of deep green coloration indicated the presence of tannin in the sample extract.

Test for terpenoids
0.5 ml extract, 2 ml of chloroform were mixed then a few drops of concentrated sulfuric acid was added. The appearance of the reddish-brown colour at the interface showed the presence of terpenoids in the sample extract.
Test for phenols
1 ml extract, 2 ml of distilled water were mixed then 2 drops of 10% FeCl₃ was added. The appearance of a blue or green colour indicated the phenol group present in the sample extract.

Test for protein
1 ml extract, 500µL of copper sulphate solution, 500µL of 5% sodium hydroxide solution were mixed. The appearance of purple violet colour indicated the presence of protein in the plant extract.

Test for carbohydrates
1-2 ml extract, 1-2 drop of α-naphthol was added and then the 2 ml of conc. sulphuric acid was added. The appearance of a violet ring indicated the presence of sugar in the plant extract.

Thin Layer Chromatographic analysis of plant extract
Thin-layer chromatography was performed to detect the chemical profile of different extract of Gymnemasylvestre. The TLC plates were prepared according to standard protocols as slightly modified, 25 g of silica gel-G was mixed with 50 ml of distilled water and with the help of spreader slurry formed uniformly spread over TLC plates with a thickness of 0.25 mm. As a stationary phase, TLC plates were allowed to dry at room temperature and then plates were incubated in an oven at 100ºC for 2 hrs. for activation. The mobile phase consists of the solvent system (selected based on the presence of phytochemicals). A soft pencil was used for TLC platemarking. Prepared plant extracts were loaded on the activated TLC plates on a marked spot by using the applicator and then allowed to run for 2 hrs (Harborne, 1998 and Mishra et al., 2014). The TLC spots were exposed in UV-illuminator to analyse the migration of band patterns and the Retention factor values (Rf) of bands were calculated and noted using the following formula-

\[
R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}
\]

Synthesis of silver nanoparticles from Gymnemasylvestre
10 ml of the stem aqueous extract of Gymnemasylvestre was added into 90 ml of an aqueous solution of 3mM silver nitrate. The mixture was incubated in a water bath (Yorco Universal) for 30 minutes at 50°C and exposed to a range of controlled temperatures for 24 hours. The appearance of brown colour in solution showed the formation of Gymnemasylvestresilver nanoparticles. The solution of silver nanoparticles was then kept in dark and dried in hot air oven to make a crystallized form for further analysis and preserved at 4 °C for further use (Supraja et al., 2017).

Characterization of silver nanoparticle
UV-Visible Spectroscopy analysis of synthesized nanoparticles
The synthesis of Gymnemasylvestresilver nanoparticles was confirmed using UV-Visible Spectrophotometer (Systronics UV-VIS Spectrophotometer 117) with a resolution between 200 and 700 nm (Supraja et al., 2017).

FTIR analysis for synthesized nanoparticles
Fourier transforms infrared (FTIR) spectroscopy is a chemical analytical tool used to detect the possible functional groups present in Gymnemasylvestre silver nanoparticles based on the peak value in the region of infrared radiation. The major function of FTIR spectroscopy was to determine the nature of the association of Phyto-molecules or Phytoextracts with nanoparticles. Infra-red (IR) analysis of Gymnemasylvestresilver nanoparticles was done with the help of Infra-red Spectrophotometer (BRUKER, ALPHA II, ECO ATR) at the Department of Chemistry, Govt. V.Y.T. PG. Autonomous College, Durg, Chhattisgarh, India.

X-Ray Diffraction analysis
Silver nanoparticles were harvested and its crystalline structure characterized by XRD analysis. X-Ray Diffraction (XRD) tool was used to evaluate the crystalline nature of synthesized silver nanoparticles of Gymnemasylvestre. The XRD pattern was recorded using computer-controlled XRD-system and analysed through PAN Analytical software. The crystallite domain size or silver nanoparticles was calculated from the width of the XRD peaks by using the following Debye-Scherrer formula-
Where $\theta$ is Bragg's angle, $\lambda$ is the wavelength of X-ray (1.5406 Å) and $\beta$ is the Full Width at Half Maximum (FWHM) (Haix and Jurado, 2001).

**Result And Discussion:**

**Qualitative phytochemical analysis**

Phytochemical study of medicinal plant is very useful for the investigation of many biologically active bioactive compounds that contribute to the colour, flavour and other features of leaves, flowers, fruits and stems (Singh and Deo, 2014). Leaf and stem extracts of *Gymnemasylvestre* were screened for the qualitative presence of major active ingredients such as alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins, terpenoids, phenols, protein and carbohydrates according to the standard method.

Saponin was found in all the sample extract. Tannin was found only in the aqueous leaf extract. The steroid was present in aqueous, methanol and petroleum ether leaf and stem extract. Flavonoid was found in Aqueous, methanol and petroleum ether leaf and stem extract along with benzene stem extract. Alkaloids were found in aqueous and benzene leaf and stem extracts along with chloroform leaf extract. Cardiac glycoside was found in aqueous, chloroform and petroleum ether leaf and stem extract along with methanol stem extract. Terpenoid was found in aqueous, methanol and chloroform leaf extract along with methanol and petroleum ether stem extract. Phenol was present in methanol and benzene leaf extract. Carbohydrate was found in methanol leaf and stem extract. Protein was found in the aqueous stem, leaf and stem methanol extract (Fig 02; Table No 01).

Many investigations reported these phytoconstituents to have many therapeutic applications, so it is expected that these species have many medicinal uses. Our findings are comparable to those obtained by Singh and Deo, 2014, who carried out preliminary phytochemical analysis using aqueous, ethanolic and methanolic leaf extract of *Gymnemasylvestre* R.Br and reported the presence of important phytoconstituents such as alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenols, saponins and tannins responsible for various medicinal and physiological properties. Similarly, Naidu *et al*., in 2013 used chloroform, hexane and methanol solvents for extraction of leaves extract and performed phytochemical screening of *Gymnemasylvestre*. The investigation revealed the presence of many important phyto compounds such as alkaloids, coumarins, flavonoids, phenols, saponins, steroids, tannins and triterpenoids. Senthilkumar, 2015 investigated preliminary phytochemical analysis and in vitro antibacterial activity of *Gymnemasylvestre*. For extraction solvents like chloroform, ethanol, ethyl acetate and hexane were used and the phytochemical study revealed the presence of many active phyto compounds such as alkaloids, carbohydrate, flavonoids, saponin, tannins and terpenoids. Arunachalam *et al*., in 2014 studied phytochemical screening of aqueous extracts of *Gymnemasylvestre* and reported that phyto compounds such as alkaloids, flavonoids, phenols, steroids, tannins and triterpenes present in leaf extract of *Gymnemasylvestre* have therapeutic importance. Our finding indicated that the stem and leaf of *Gymnemasylvestre* exhibited the presence of many important secondary metabolites useful for various therapeutic and medicinal properties.

**Thin Layer Chromatographic analysis of plant extract**

TLC study revealed the presence of various active biomolecules in the leaf extract. TLC profiling of the plant sample has been carried out with different bands. TLC chromatogram for saponins showed the separation of four fractions ($R_f = 0.36, 0.90, 0.82, 0.33$), for cardiac glycoside two fractions ($R_f = 0.40, 0.96$) and for flavonoid two fractions ($R_f = 0.71, 0.92$) under visible light. The sample concentration of 10 μl was found to be showing more bands in case of saponins and cardiac glycosides whereas 5 μl of sample concentration was sufficient to generate the bands in case of flavonoids (Fig- 03 and Table No. 02).

Thin layer chromatographic study provides information about polarity of many phytoconstituents present in an extract with $R_f$ values. Subashini *et al*., 2015 evaluated thin layer chromatographic study of methanol extract of *Gymnemasylvestre*. TLC study revealed $R_f$ values of 0.23, 0.35, 0.45, 0.59, 0.69 and 0.85 by using solvents system of Chloroform: Methanol (9:1).
Synthesis of silver nanoparticles from Gymnemasyvulstre

The silver nanoparticles were synthesized using an aqueous extract of stem of Gymnemasyvulstre. UV-Visible spectral analysis showed an absorbance peak at 430 nm with special reference to the excitation of surfaces plasmon vibration by silver nanoparticles. FTIR analysis of nanoparticles revealed the presence of molecular functional groups such as amides, amines, phenolic compounds, and alkenes. These phytochemicals act capping and stabilizing agents for silver nanoparticles. XRD patterns also suggested the occurrence of crystalline silver ions.

UV-Visible analysis of synthesized Gymnemasyvulstre AgNPs

UV-vis absorption spectra of colloidal solution of stem aqueous extract and silver nitrate solution have been depicted in Fig- 04. Originally the silver nitrate solution was colourless but after addition of aqueous stem extract, it turned as brown. Change in colour of the solution from colourless to brown strongly indicated the synthesis of silver nanoparticles in stem extract medium. The colour of the solution was varied with the increasing incubation time as colourless, yellowish-brown, brown and deep brown. The increase of incubation period increases the reduction rate of silver ions, this gives a higher concentration of silver nanoparticles have a deep brown solution. A single and strong absorption spectrum at 419 nm is characteristics surface plasmon resonance (SPR) peak of silver nanoparticles and hence it confirmed their formation. The strong and sharp absorption spectra are because of surface plasmon vibrations of silver nanoparticles in the visible region.

FTIR Spectral analysis of Gymnemasyvulstre AgNPs

FTIR spectrum is a very useful tool for the discovery of the possible bio-molecules interactions in the silver nanoparticles of stem extract of Gymnemasyvulstre. FTIR spectrum was applied in the range of 400 - 4000 cm⁻¹. The wide-ranging infrared spectrum of aqueous stem extract of Gymnemasyvulstre has been presented in Fig- 05 & Table No. 03. Characteristic absorption bands were exhibited at 3248.44 cm⁻¹ for alcohols and phenols (O-H stretching), 2929.17 cm⁻¹ for methylene groups of the protein or alkenes(C-H stretching), 1630.05 cm⁻¹ for alkene or amide (C=C stretching), 1334.03 cm⁻¹ for amines and amides (C-N stretching), 1190.83 cm⁻¹ for amines and amides (C-N stretching), 879.90, 826.25 cm⁻¹ for alkenes (C-H stretching), 741.30 cm⁻¹ aromatic compounds (C-H stretching) and 655.11 cm⁻¹ for halo compounds (C-Br stretching).

Similarly, FTIR spectrum analysis of silver nanoparticles synthesized from leaves of Gymnemasyvulstre was studied in previous investigations and reported absorption bands at 617.3 cm⁻¹ for C-C bending, 824.9 cm⁻¹ for N-H deformation of amines, 1050 cm⁻¹ for C-N stretching of aliphatic amines (Gomathiet al., 2020). Supraja et al., 2017 investigated FTIR spectrophotometric analysis of silver nanoparticle of Gymnemasyvulstre and reported wide-ranging infrared spectrum. The characteristic absorption bands were exhibited at 3355, 2989, 2885, 2822, 2104, 1772, 1637, 1406 and 1049 cm⁻¹ correspond to the functional groups such as primary and secondary amines, group of proteins (N-H stretching vibration), alkenes (C-H stretching vibration), alkanes (C-H stretching), aldehydes (C-H stretching), alkynes (C=C stretching), carbonyls in proteins (C=O stretching), primary amines (N-H stretching), aromatics (C-C stretching) and aliphatic amines (C-C stretching) respectively.

Our results from FTIR spectral analysis suggested that phytochemicals like flavonoids, terpenoids and polyphenols are found in the stem extract were mainly responsible for the reduction process of silver ions into nanoscale silver particles and protein present in extract could act as capping agent that help in the stabilization the silver nanoparticles of stem extract of Gymnemasyvulstre. Previous research works made by Supraja et al., 2017 and Gomathiet al., 2020 may be correlate with our observations and also supported our findings.

XRD analysis of Gymnemasyvulstre AgNPs

For confirming the synthesis of silver nanoparticle as a crystalline form, the XRD study was performed with the diffraction from 10° to 80° at 20 angles. Figure 06 showed the XRD graph of Gymnemasyvulstre silver nanoparticles. X-Ray Diffraction study provides various size-dependent features of crystalline nature of silver nanoparticles like height, width and peak position. Four different and important characteristic peaks were observed at the 20 of 23.59°, 27.99°, 32.26° and 46.23° that correspond to (548), (967), (2425) and (1123) heights indicating that SNPs are highly crystalline respectively. In XRD pattern analysis a peak at 32.26° exhibited the synthesis of pure silver nanoparticles. Crystalline size of the silver nanoparticles was determined by the help of Scherrer’s formula and found to be 39.5 nm.

Similarly, Supraja et al., 2017 characterized silver nanoparticle with the help of XRD analysis and reported that silver nanoparticles synthesized from leaves extract of Gymnemasyvulstre were highly crystalline. Investigation
revealed that five important and different characteristic peaks were observed at the 2θ of 38.6°, 45.8°, 58.7°, 64.8° and 78.6° correspond to (111), (200), (220), (311) and (222) respectively. Gomathiet et al., 2020 studied XRD crystalline analysis of silver nanoparticle of Gymnemasty lentre and observed three different and important characteristic peaks at the 2θ of 45, 65 and 77 correspond to (200), (220) and (222) planes of Ag crystal. The study revealed that the mean particle size of silver nanoparticles decreases from 28 nm to 23 nm about a rise in extract volume from 5 to 15 ml. Pingale et al., 2018 performed the plant-mediated synthesis of silver nanoparticles from aqueous extract of stem and root of Gymnemasty lentre and found that the diffraction peaks of the range of 2θ (20 – 80°) correspond to (111), (200), (220) and (311) planes and average crystalline size of silver nanoparticles was 25.3 nm. Thus by and large our findings are affirmative to the previous author and authenticate the pharmaceutical features of Gymnemasty lentre for further pharmacological applications.

Table No 01: Showing qualitative phytochemical analysis of leaf and stem extract of Gymnemasty lentre.

<table>
<thead>
<tr>
<th>Phytocompounds</th>
<th>Distilled water</th>
<th>Methanol</th>
<th>Chloroform</th>
<th>Benzene</th>
<th>Petroleum ether</th>
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<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Stem</td>
<td>Leaf</td>
<td>Stem</td>
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<tr>
<td>Steroid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Cardiac Glycoside</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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</tr>
<tr>
<td>Terpenoid</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Protein</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Phenol</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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</tr>
<tr>
<td>Carbohydrate</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

(+) – present; (-) - absent
**Figure 02:** Showing qualitative phytochemical analysis of stem and leaf extracts of *Gymnemasyville*. 

*Distilled water extract*
**Figure 03:** Showing TLC Chromatogram of stem and leaf extract of *Gymnemasisvestre*.
*a.* methanol leaf extract, *b.* methanol stem extract, *c.* chloroform leaf extract
*d.* chloroform stem extract, *e.* petroleum ether leaf extract, *f.* petroleum ether stem extract, *g.* benzene leaf extract and *h.* benzene stem extract.

**Table No 02:** Showing Rf Value of TLC Chromatogram of plant extract of *Gymnemasisvestre*.

<table>
<thead>
<tr>
<th>S No.</th>
<th>Sample</th>
<th>Max no. of bands</th>
<th>Rf Value</th>
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<tbody>
<tr>
<td>1.</td>
<td>Methanol leaf extract</td>
<td>2</td>
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</tr>
<tr>
<td>2.</td>
<td>Methanol stem extract</td>
<td>2</td>
<td>0.90</td>
</tr>
<tr>
<td>3.</td>
<td>Chloroform leaf extract</td>
<td>3</td>
<td>0.40</td>
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<tr>
<td>4.</td>
<td>Chloroform stem extract</td>
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<td>0.96</td>
</tr>
<tr>
<td>5.</td>
<td>Petroleum ether leaf extract</td>
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<td>0.71</td>
</tr>
<tr>
<td>6.</td>
<td>Petroleum ether stem extract</td>
<td>1</td>
<td>0.92</td>
</tr>
<tr>
<td>7.</td>
<td>Benzene leaf extract</td>
<td>1</td>
<td>0.82</td>
</tr>
<tr>
<td>8.</td>
<td>Benzene stem Extract</td>
<td>2</td>
<td>0.33</td>
</tr>
</tbody>
</table>

**Figure 04:** Showing UV-Visible Spectrum of stem extract silver nanoparticles of *Gymnemasisvestre* 3mM AgNO₃.

**Table No 03:** Showing Functional groups present in *Gymnemasisvestre*AgNPs.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Absorption Spectra (cm⁻¹)</th>
<th>Probable Fuctional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3248.44</td>
<td>O–H stretching of the alcohols and phenols</td>
</tr>
<tr>
<td>2.</td>
<td>2929.17</td>
<td>C–H stretching of methylene groups of the protein or alkenes</td>
</tr>
<tr>
<td>3.</td>
<td>1630.05</td>
<td>C=C stretching of alkene and amide</td>
</tr>
<tr>
<td>4.</td>
<td>1334.03</td>
<td>C=N stretching of amines and amides</td>
</tr>
<tr>
<td>5.</td>
<td>1190.83</td>
<td>C=N stretching of amines and amides</td>
</tr>
<tr>
<td>6.</td>
<td>879.90</td>
<td>C-H stretching of alkenes</td>
</tr>
<tr>
<td>7.</td>
<td>826.25</td>
<td>C-H stretching of alkenes</td>
</tr>
<tr>
<td>8.</td>
<td>741.30</td>
<td>C-H stretching of aromatic compounds</td>
</tr>
<tr>
<td>9.</td>
<td>655.11</td>
<td>C-Br stretching of halo compounds</td>
</tr>
</tbody>
</table>
Conclusion: -
In the present study, we have successfully screened qualitative phytochemical analysis and synthesized the green silver nanoparticles from *Gymnemasylvestre* stem extract. The phytochemical analysis of stem and leaves extract showed the presence of important secondary metabolites such as alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins, terpenoids, carbohydrates, phenols, and proteins. The biologically synthesized silver nanoparticles were characterized through UV-Visible Spectroscopy, FTIR analysis, and XRD analysis. Bioactive secondary metabolites found in the aqueous extract of *Gymnemasylvestre* have to reduce and capping properties that’s why aqueous extract is suitable for green synthesis of silver nanoparticles within small times of 30 minutes. UV-Visible spectrum analysis confirmed the optical properties of nanoparticles based on surface plasmon resonance (SPR) responsible for the colour change of the medium. FTIR analysis confirmed the presence of specific functional groups such as alkenes, amines, amides, alcohols, phenols, aromatic and halo compounds.
Crystalline nature and size of silver nanoparticles were determined by the help of XRD pattern. XRD analysis showed the size of silver nanoparticles was 39.5 nm obtained by Scherrer’s formula.

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References: