Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/11916
DOI URL: http://dx.doi.org/10.21474/IJAR01/11916

ISSN NO. 2320-5407

RESEARCH ARTICLE

PHARMACOLOGICAL AND NANOBIOTECHNOLOGICAL ASPECTS OF *GYMNEMA SYLVESTRE*

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Manuscript Info

Manuscript History
Received: 25 August 2020
Final Accepted: 28 September 2020
Published: October 2020

Key words:-
Gymnemasylvestre, Phytocompounds, Nanoparticles, FTIR, XRD

Abstract

*Gymnemasylvestre*commonly knownGurmar orsugar destroyer, belonging to the family Asclepiadaceae, is a slow-growing,woody climberdistributed throughout India, traditionally used in Ayurvedic system of medicine forthe 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 extractof *Gymnemasylvestre*was utilized for the synthesis of silver nanoparticles. UV-Vis spectrophotometric studyexhibited absorbance peak at 419 nm that measures the reduction ofsilver nitrate tosilver 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 of39.5 nm by Scherrer's formula. The presence of this phytoconstituent suggested vital medicinal featuresof theplant and their importancein 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 ingredientsfor 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 availabilityto 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 indigenousmedicinal flora that has been used in Indian traditional medications from the pre-historic 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 andGurmar in Hindi which means "destroyer of sugar" (Mitra*et al*., 1995; Kumar, 2015).*Gymnemasylvestre* is also being widely used forthe 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*

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Gentianales
Family	Asclepiadaceae
Genus	*Gymnema*
Species	*sylvestre* (Duke *et al*., 1997; Kritikar and Basu, 1999)

Vernacular name of *Gymnemasylvestre*

Language	**Vernacular Name**
Arabic	Barkista
Bengali	Meraasingi
English	Periploca of the woods
Hindi	Gurmar
Malayalam	Cakkarakkoli
Marathi	Kavali
Sanskrit	Madhunashini
Tamil	Sirukurunjan
Telugu	Podapatra
Tibetan	Sasinga
Urdu	Gurmar (Jayachitra and Muniyandi, 2015)

Figure 01:- *Gymnemasylvestre*.

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 *Gymnemasylvestre*small 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 *Gymnema*generally 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 leafand stem of *Gymnemasylvestre*were rinsed thrice with distilled water followed by double distilled water to remove the dust particles and other contaminants then after thatshade dried at 25- 30 ℃ 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℃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 ℃ 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 weremixed then 2 drops of 10% $FeCl_3$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-naphthalol 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 Chromatographicanalysis of plant extract
Thin-layer chromatography was performed to detect the chemical profile of different extract of *Gymnemasylvestre*. The TLC plates were preparedaccording 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. Asa 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 onthe 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-

$$R_f = \frac{\textbf{Distance travelled by solute}}{\textbf{Distance travelled by solvent}}$$

Synthesis of silver nanoparticlesfrom*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 24hours. The appearance of brown colour in solution showed the formation of *Gymnemasylvestre*silver 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 *Gymnemasylvestre*silver nanoparticles was confirmed using UV-Visible Spectrophotometer (Systronics UV-VIS Spectrophotometer 117) with a resolutionbetween 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 nanoparticlesbased 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 *Gymnemasylvestre* silver nanoparticles was done with the help ofInfra-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) toolwas 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-

$$D = \frac{0.94\lambda}{\beta \cos\theta}$$

Where θ is Bragg's angle,λ is the wavelength of X-ray(1.5406 Å) and β 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 withbenzene stem extract. Alkaloids werefound in aqueous and benzene leaf and stem extracts along with chloroform leaf extract. Cardiac glycoside wasfound 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 preliminaryphytochemical 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 tanninsresponsible for various medicinal and physiological properties.Similarly, Naidu*et al*., in 2013 used chloroform, hexane andmethanol solvents for extraction of leaves extract and performed phytochemical screening of *Gymnemasylvestre*.The investigation revealed the presence of many important phytocompounds 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 phytocompounds such as alkaloids, carbohydrate, flavonoids, saponin, tannins and terpenoids.Arunachalam*et al*., in 2014 studied phytochemical screeningof aqueousextracts of *Gymnemasylvestre* and reported that phytocompounds such as alkaloids,flavonoids,phenols,sterols,tannins and triterpenes present in leaf extract of *Gymnemasylvestre* have therapeuticimportance.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 10µl was found to be showing more bands in case of saponins and cardiac glycosides whereas 5µl 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 Rf values. Subashini*et al*., 2015 evaluatedthin layerchromatographic study of methanol extract of *Gymnemasylvestre*. TLC study revealed Rf values of0.23, 0.35, 0.45, 0.59, 0.69and 0.85 by using solventsystem of Chloroform: Methanol (9:1).

Synthesis of silver nanoparticles from*Gymnemasylvestre*

The silver nanoparticles were synthesized using an aqueous extract of stem of *Gymnemasylvestre*. 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*Gymnemasylvestre*AgNPs

UV-vis absorption spectra of colloidal solution of stem aqueous extract and silver nitrate solution have been depicted inFig- 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 *Gymnemasylvestre*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 *Gymnemasylvestre*. FTIR spectrum was applied in the range of 400 - 4000 cm^{-1}.The wide-ranging infrared spectrum of aqueous stem extract of *Gymnemasylvestre*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), 1630.05cm^{-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, 826.25cm^{-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 *Gymnemasylvestre* was studied in previous investigations and reported absorption bands at 617.3 $cm^{-1}$for C-C bending, 824.9cm^{-1} for N-H deformation ofamines, 1050cm^{-1} for C–N stretching of aliphaticamines (Gomathi*et al*., 2020).Supraja*et al*., 2017 investigated FTIR spectrophotometric analysis of silver nanoparticle of *Gymnemasylvestre* and reported wide-ranging infrared spectrum. The characteristic absorption bands were exhibited at 3355, 2989, 2885, 2822, 2104, 1772, 1637, 1406 and 1049cm^{-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), 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 *Gymnemasylvestre*. Previous research works made bySuprajaet *al*., 2017 and Gomathi*et al*., 2020 may be correlative with our observations and also supported our findings.

XRD analysis of*Gymnemasylvestre*AgNPs

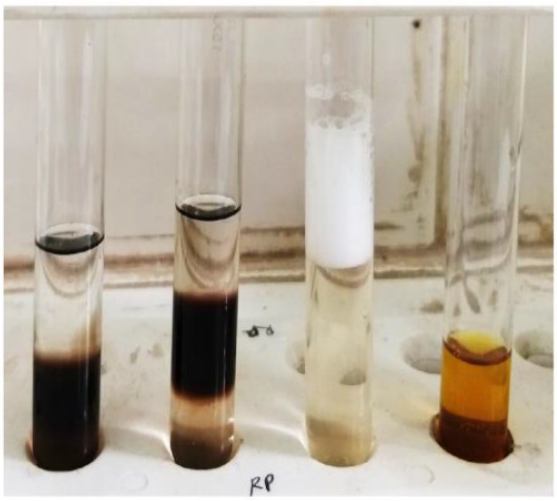
For confirming the synthesis of silver nanoparticle as a crystalline form, the XRD study was performed with the diffraction from 10° to80° at 2θ angles.Figure 06 showed the XRD graph of *Gymnemasylvestre* silver nanoparticles. X-Ray Diffraction study provides various size-dependent features of crystalline nature of silver nanoparticles like-height, width and peak position. Four different and important characteristic peaks were observed at the 2θ 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 foundto 39.5 nm.

Similarly, Suprajaet *al*., 2017 characterized silver nanoparticle with the help of XRD analysis and reported that silver nanoparticles synthesized from leaves extract of *Gymnemasylvestre* were highly crystalline. Investigation

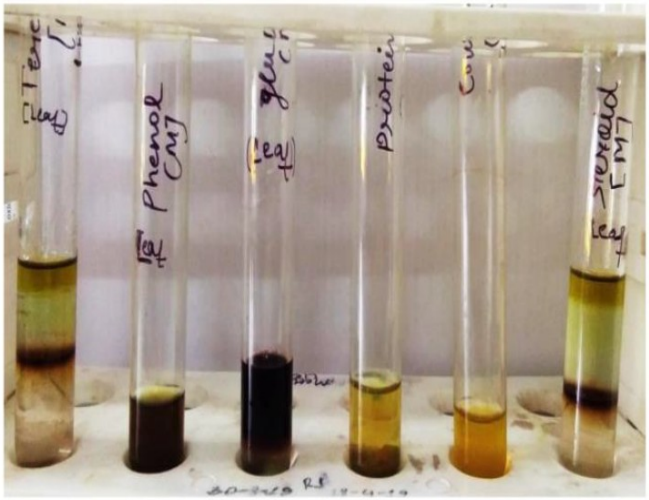
revealed that five important and different characteristic peaks were observedat the 2θ of38.6°, 45.8°, 58.7°, 64.8° and 78.6°correspond to(111), (200), (220), (311) and (222) respectively.Gomathi*et al*., 2020studied XRD crystalline analysis of silver nanoparticle of *Gymnemasylvestre*and observed three different and important characteristic peaks at the 2θ of45, 65and 77correspond to(200), (220) and(222) planes of Ag crystal. The study revealed that the mean particle size of silver nanoparticles decreases from 28 nm to23 nm abouta 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 *Gymnemasylvestre* and found that the diffraction peaks of therange 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 *Gymnemasylvestre*for further pharmacological applications.

Table No 01:- Showing qualitative phytochemical analysis of leaf and stem extract of *Gymnemasylvestre*.

Phytocompounds	Solvent system									
	Distilled water		Methanol		Chloroform		Benzene		Petroleum ether	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Steroid	+	+	+	+	-	-	-	-	+	+
Saponins	+	+	+	+	+	+	+	+	+	+
Tannins	+	-	-	-	-	-	-	-	-	-
Flavonoid	+	+	+	+	-	-	-	+	+	+
Alkaloid	+	+	-	-	+	-	+	+	-	-
Cardiac Glycoside	+	+	-	+	+	+	-	-	+	+
Terpenoid	+	-	+	+	+	-	-	-	-	+
Protein	+	+	-	+	-	-	-	-	-	-
Phenol	-	-	+	-	-	-	+	-	-	-
Carbohydrate	-	-	+	+	-	-	-	-	-	-
(+) – present; (-) - absent										



Benzene extract



Methanol extract

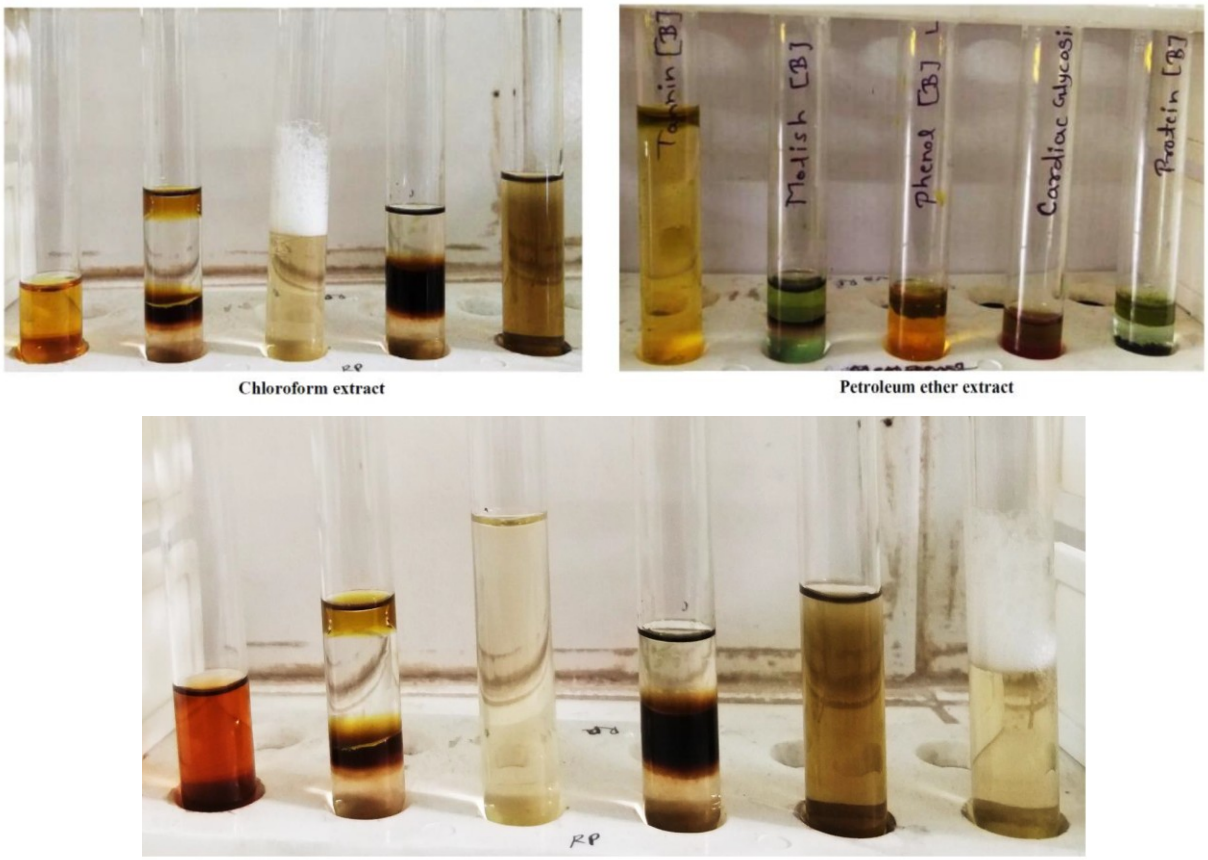


Figure 02:-Showing qualitative phytochemical analysis of stem and leaf extracts of *Gymnemasylvestre*.

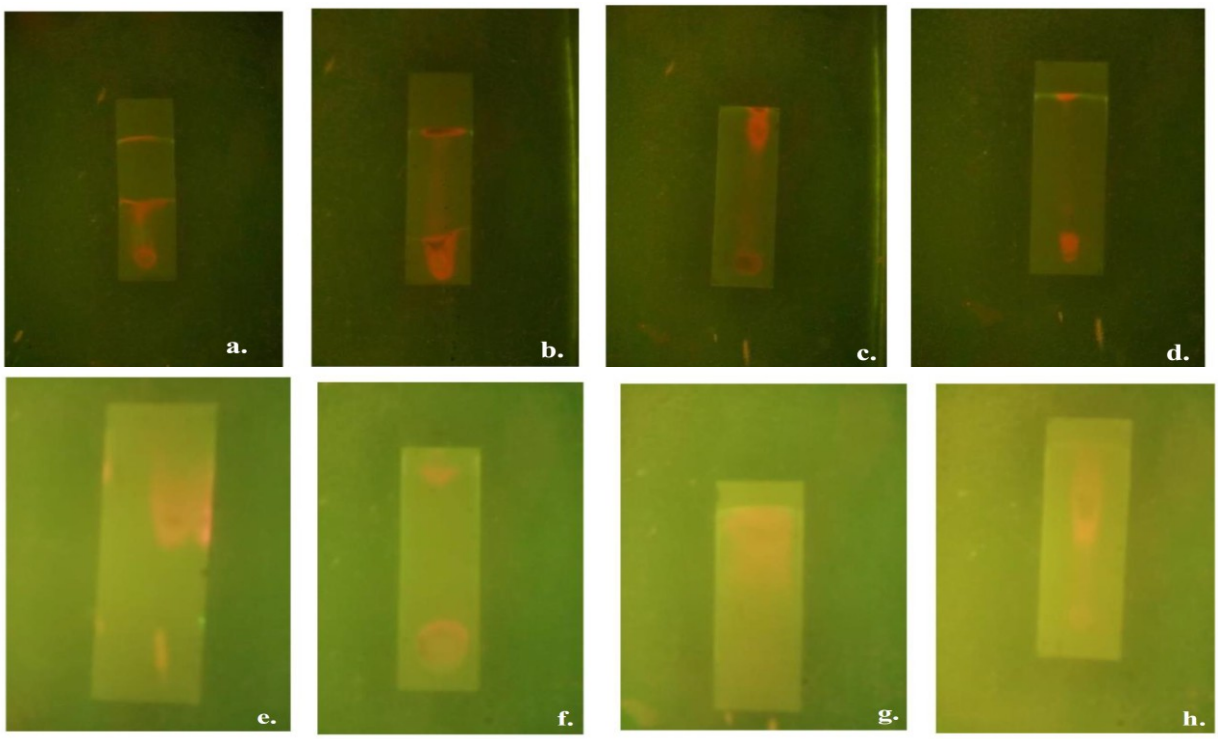


Figure 03: Showing TLC Chromatogram of stem and leaf extract of *Gymnemasylvestre*.
a. methanol leaf extract, **b.** methanol stem extract, **c.** chloroform leaf extract
d. chloroform stem extract, **e.** petroleum ether leaf extract, **f.** petroleum ether stem extract, **g.** benzene leaf extract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

Table No 02:- Showing R_f Value of TLC Chromatogram of plant extract of *Gymnemasylvestre*.

S No.	Sample	Max no. of bands	R_f Value
1.	Methanol leaf extract	2	0.36
2.	Methanol stem extract	2	0.90
3.	Chloroform leaf extract	3	0.40
4.	Chloroform stem extract	1	0.96
5.	Petroleum ether leaf extract	2	0.71
6.	Petroleum ether stem extract	1	0.92
7.	Benzene leaf Extract	1	0.82
8.	Benzene stem Extract	2	0.33

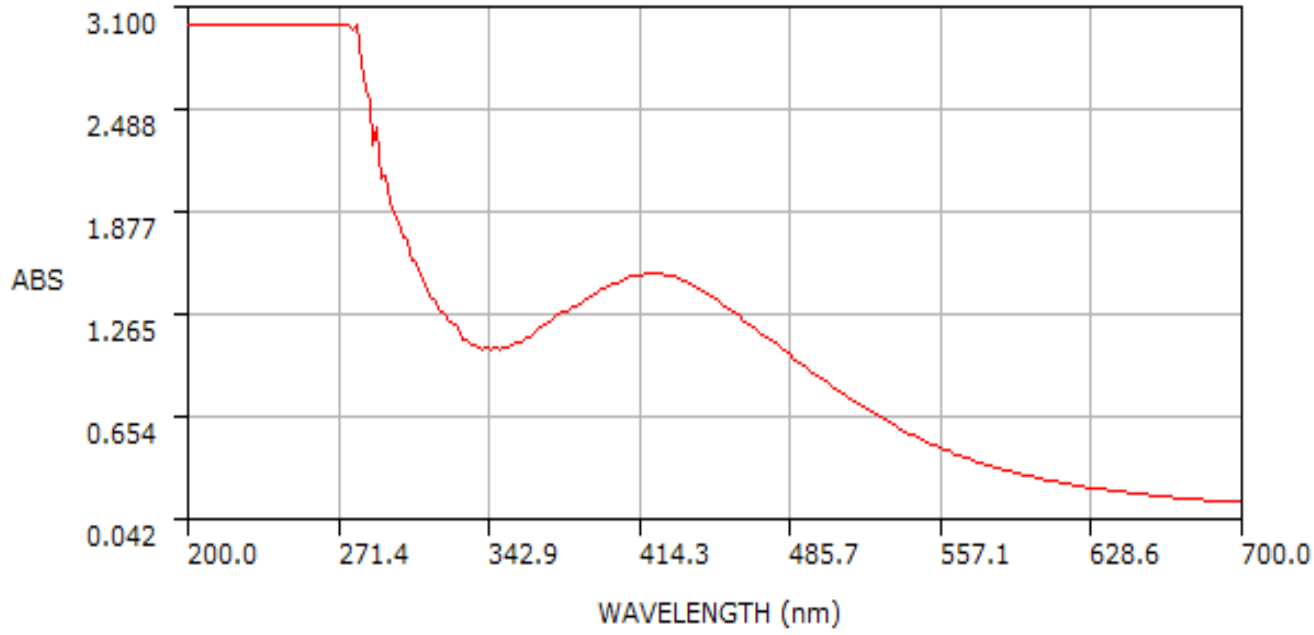


Figure 04:-Showing UV-Visible Spectrum ofstem extract silver nanoparticles of*Gymnemasylvestre*at 3mM $AgNO_3$.

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

S. No.	Absorption Spectra (cm^{-1})	Probable Fuctional groups
1.	3248.44	O–H stretching of the alcohols and phenols
2.	2929.17	C–H stretching of methylene groups of the protein or alkenes
3.	1630.05	C=C stretching of alkene and amide
4.	1334.03	C-N stretching of amines and amides
5.	1190.83	C-N stretching of amines and amides
6.	879.90	C-H stretching of alkenes
7.	826.25	C-H stretching of alkenes
8.	741.30	C-H stretching of aromatic compounds
9.	655.11	C-Br stretching of halo compounds

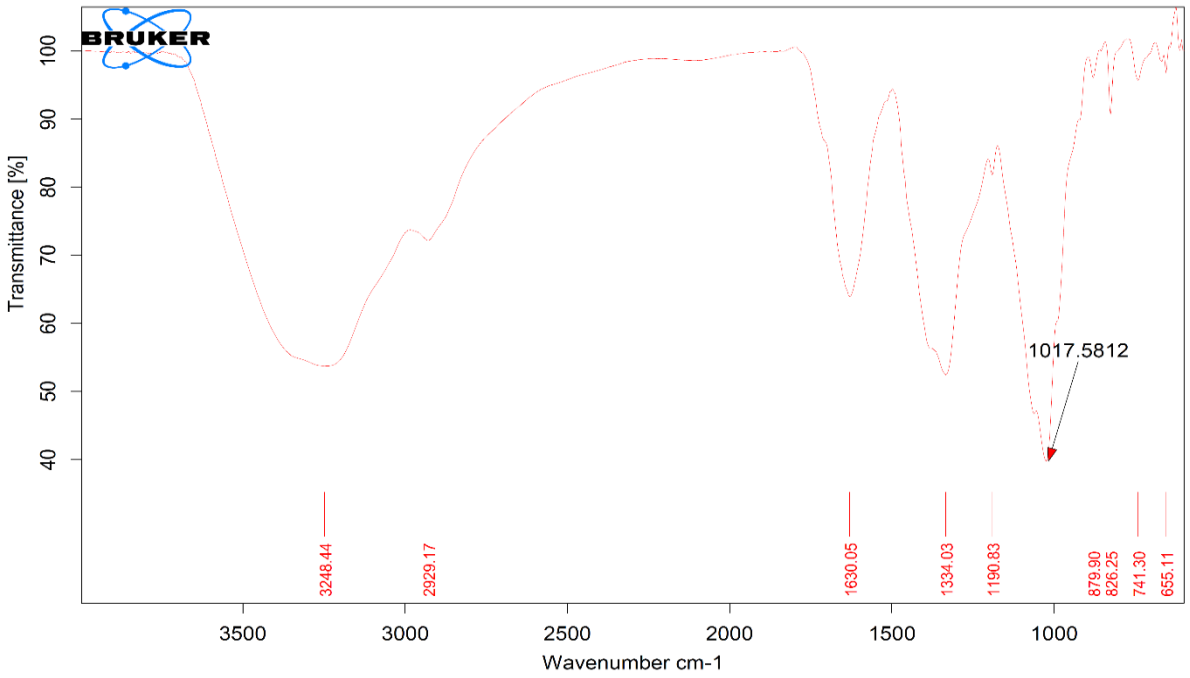


Figure 05:- Showing FTIR Spectrum of stem extract silver nanoparticles of*Gymnemasylvestre*.

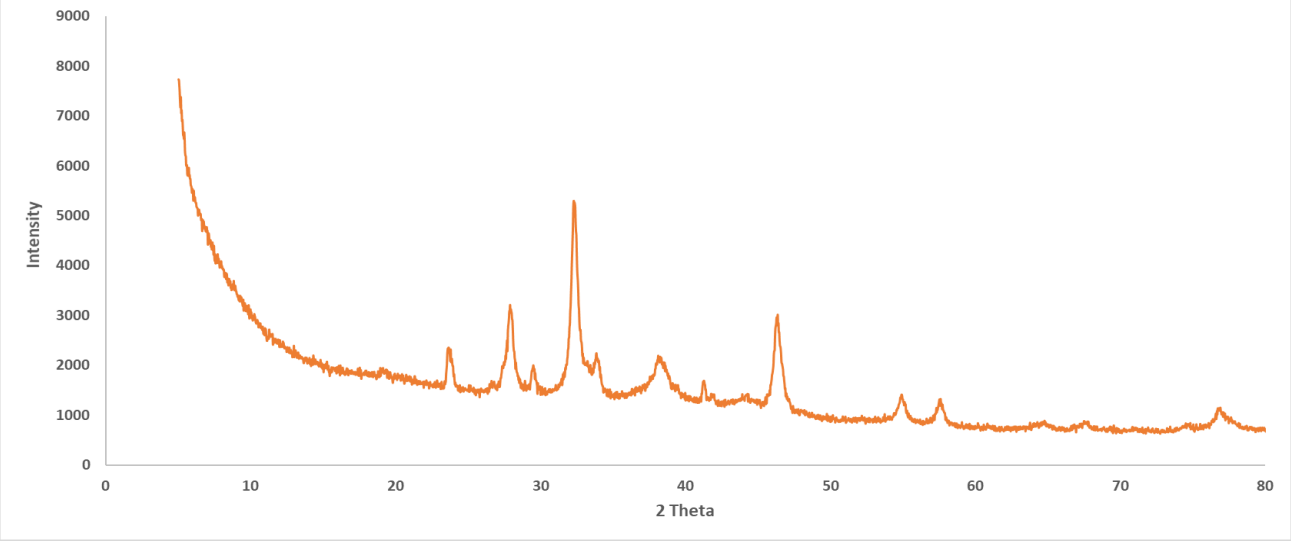


Figure 06:-Showing XRD- Spectra of stem extract silver nanoparticles of*Gymnemasylvestre*.

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 nanoparticleswere characterized through UV-Visible Spectroscopy, FTIR analysis, andXRD analysis. Bioactive secondary metabolites found in the aqueous extract of *Gymnemasylvestre*have to reduce and caping 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.

Acknowledgement:-

The authors are thankful to Council of Scientific and Industrial Research, New Delhi, for the award of Junior Research Fellowship for the study.

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