PRELIMINARY EVALUATION OF GYMNEMA SYLVESTRE EXTRACT-BASED NANONEUTRACEUTICALS

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KEYWORDS

Gymnema Sylvestre, Nanoneutraceuticals, Phytochemical Screening, Column Chromatography, TLC Analysis, Pharmacological Potential, etc

ABSTRACT

Promising phytochemical and physicochemical characteristics were found in early assessment of Gymnema Sylvestre extract-based nanoneutraceuticals. By means of evaluations of foreign organic matter, ash values, and extractive values, all within reasonable limits, standardizing investigations verified the authenticity and purity of the plant material Thin Layer Chromatography (TLC) study further corroborated phytochemical screening results confirming the presence of flavonoids, alkaloids, triterpenoids, and steroids. While spectroscopic methods gave structural understanding of the bioactive chemicals, column chromatography made active fractions possible to separate. These results imply that Gymnema Sylvestre extract has bioactive components with pharmacological significance, hence having great potential for nanoneutraceutical formulations. Investigating its medicinal uses and safety profile will need further research.

1. Introduction

The growing interest in nanoneutraceuticals stems from their potential to enhance the bioavailability, stability, and therapeutic efficacy of bioactive compounds derived from natural sources. Gymnema sylvestre, a well-known medicinal plant, has been extensively studied for its antidiabetic, antioxidant, and anti-inflammatory properties. [1-6] The plant's bioactive constituents, including gymnemic acids and flavonoids, play a crucial role in regulating blood glucose levels, making it a promising candidate for diabetes management. However, the poor aqueous solubility, low bioavailability, and stability issues associated with Gymnema sylvestre extract (GSE) pose significant challenges for its clinical application.

Nanotechnology-based delivery systems offer an effective strategy to overcome these limitations by improving solubility, controlled release, and absorption of the extract. The incorporation of GSE into nanoneutraceutical formulations can facilitate targeted and sustained drug delivery, ensuring better therapeutic outcomes. Therefore, preliminary evaluation of these nanoneutraceuticals is essential to assess their physico-chemical properties, encapsulation efficiency, drug loading, release profile, and stability before advancing to in vivo and clinical studies. [7-10]

This study aims to develop and characterize Gymnema sylvestre extract-based nanoneutraceuticals by evaluating their solubility, thermal stability, crystallinity, entrapment efficiency, and drug release behavior. The findings from this research will provide fundamental insights into the potential of nanoneutraceutical formulations in optimizing the therapeutic efficacy of GSE, particularly for metabolic disorders such as diabetes.

2. Materials and Method

A. Materials

1. Chemicals and Regents

The solvent methanol, chloroform, acetonitrile, Petroleum ether, ethanol, Chemicals were obtained from Sigma Chemical Company (Sigma-Aldrich, St. Louis MO,USA).

2. Equipment's

The study utilized various laboratory instruments for formulation and evaluation. An electronic weighing balance (Shimadzu, Japan) was used for accurate measurement of sample weights. The pH meter (Lab India, Mumbai) was employed to determine the pH of the formulations. A 1H-NMR BRUKER 400MHz NMR, while an FT-IR Spectrophotometer (Jasco-4600) was used for functional group analysis of the extract. The study utilized a chromatographic column (40 cm × 3.5 cm), a separating funnel, cotton, an iodine chamber, TLC plates with a developing chamber, and capillary tubes. Silica gel (60-120#) and organic solvents were used for separation, with n-hexane for column packing.

B. Method [11-20]

1. Collections and Drying

Whole plant extract of Gymnema Sylvestre dry extract was procured from sun pure extracts private limited. Dried plant material extract powders were passed through 120mesh to remove fine powders and coarse powder was used for extractions.

2. Authentication

The plant was authenticated department of Botany, School of life sciences, S. R. T. M. U., Nanded by comparing morphological features.

3. Standardization of plants materials:

Standardization of plants material of authenticated plants shall be done by using following parameters. Each monograph contains detailed botanical, macroscopic and microscopic descriptions with detailed illustrations and photographic images which provide visual documentation of accurately identified material. A microscopic analysis assures the identity of the material and as an initial screening test for impurities.

4. Determination of foreign organic matter

Principal:

Herbal drugs should be prepared from the confirmed part of the plant. They should be totally free from insects or moulds, including visible and excreta contaminant such as stones, sand, harmful and poisonous foreign matter and chemical residues. Animal objects such as insects and invisible microbial contaminants, which produces toxins, as well as the potential contaminants of herbal medicines. Macroscopic evaluation can easily use to determine the presence of foreign matter, although microscopy is essential in certain special cases for example starch intentionally added to "dilute" the plant material.

1. 5 gm of air dried coarsely powdered drug was spreader in a thin layer.
2. The sample was inspected with the unaided eye or with the use of 6X lens.
3. The foreign organic matter was separated manually as completely as possible.

4. Sample was weighed and percentage of foreign organic matter was determined from the weight of the powder taken.

5. Determination of moisture content

Procedure:

a) Accurately weighed glass-stopper, shallow weighing bottle, was dried.
b) 2gm of sample was transferred to the bottle and covered, the weight was taken, and sample was distributed evenly and poured to a depth not exceeding 10 mm.
c) Then loaded bottle was kept in an oven and was removed.
d) The sample was dried to constant weight.
e) After drying it was collected to room temperature in a desiccator.
f) Weighed and the loss on drying was calculated in terms of percent w/w.

6. Ash value

Ash value is used to determine quality and purity of crude drug. Ash value contains inorganic radicals like phosphates carbonates and silicates of sodium, potassium, magnesium, calcium etc. sometimes inorganic variables like calcium oxalate, silica, carbonate content of the crude drug affects 'Total Ash Value'. Such variables are then removed by treating with acid and then acid insoluble ash value is determined

The ash remaining following ignition of medicinal plant materials is determined by three different methods which measure total ash, acid-insoluble ash and water-soluble ash. The total ash method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non- physiological" ash, which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

a. Determination of Total ash

Procedure:

1. Accurately weighed 2gm of air-dried crude drug was taken in a tarred silica dish and incinerated at a temperature not exceeding 45^0C until free from carbon, cooled and weight was taken.
2. The percentage of ash was calculated with reference to the air-dried powder.

b. Determination of Water- soluble ash

Procedure:

1. The ash was obtained as per method described above and boiled for 5 minutes with 25 ml of water, filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding $45^{0\circ}$C and weight was taken.
2. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash.
3. The percentage of water soluble ash was calculated with reference to the air-dried drug.

7. EXTRACTION [21-22]

Pharmaceutical extraction is the selective solvents' separation of desired metabolites from plant or animal tissues. For either internal or external application, extracted materials might be powders,

semisolids, or liquids. Solute dissolution, diffusion, solvent penetration, and collection make up the process. Solvent type, particle size, solvent-to-–solid ratio, temperature, and duration all affect extraction efficiency. Common solvents are alcohols (EtOH, MeOH). Long hours and high temperatures help in extraction, but too high values may harm components. Classic processes (maceration, percolation, reflux) require more solvents and time whereas pressurized liquid extraction (PLE), supercritical fluid extraction (SFC), and microwave-assisted extraction (MAE) offer improved efficiency with less solvent consumption.

Methods of Extraction of Medicinal Plants

- Maceration
- Infusion
- Digestion
- Decoction
- Percolation
- Hot Continuous Extraction (Soxhlet)
- Aqueous Alcoholic Extraction by Fermentation
- Counter-current Extraction

❖ **Continuous hot Soxhlet extraction.**

Solvents

- Using different solvents in increasing order of polarity.
- Petroleum ether (60-80), ethanol and water.
- The extraction was carried out in several batches. The extraction was carried out in Soxhlet extractor till all the constituents were extracted.
- The completion of extraction was indicated by taking sample of siphon tube on TLC plate and placing it in iodine chamber.
- Absence of colored spot on plate indicated complete extraction.
- After completion of extraction, solvent was distilled off and concentrated extract was air-dried.
- The extract was stored in airtight container.
- The same procedure was followed during extraction with other solvents.
- After petroleum ether, Ethanol (95 %) extraction the exhausted marc was kept in oven to remove the solvent completely.

c. Finally, the new dried powdered material was refluxed for about 3 hours with distilled water to obtain aqueous extract. This method is used for the powder extractions.

c. Determination of Acid -insoluble ash

Procedure:

- The ash was obtained as per method described above and boiled for 5 minutes with 25 ml of 2M hydrochloric acid, filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited cooled in a desiccator and weighed.
- The percentage of acid –insoluble ash was calculated with reference to the air-dried drug.

d. Extractive values

Different extractive values like alcohol soluble extractive, water soluble extractive values were performed by standard method.

A. Determination of water-soluble extractive value

Procedure:

- 5gm of air dried coarsely powdered drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allowed to stand for 18 hours.
- Then it was filtered, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105^0c and weighed.
- Percentage of water-soluble extractive value was calculated with reference to air-dried drugs.

B. Determination of Alcohol-soluble extractive value

- 5gm of air-dried coarsely powdered drug was macerated with 100 ml of ethanol of specified strength in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allows standing for 18 hours.
- Then it was filtered, during filtration precaution was taken against loss of ethanol, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105^0c and weighed.
- Percentage of ethanol soluble extractive value was calculated with reference to air-dried powder.

❖ **Preliminary Phytochemical Screening for Various Extracts:**

The medicinal importance of a plant is due to the presence of some special substances like Alkaloids, Triterpenoids, Glycosides, Saponins, Tannins phenols, Flavonoids and Steroids, etc. The active principles usually remain concentrated in the storage organs of the plants. Considering all these facts, the present study was designed to investigate the presence of various phytochemicals in the five different extracts of whole plant which evokes various therapeutic effects.

Extracts were tested for the presence of active principles such as Alkaloids, Triterpenoids, Tannins phenols, Flavonoids and Steroids, etc. Following standard procedures were used. The most of powder contain definite chemical constituents to which their pharmacological and Biological activity depended. Qualitative chemical test used to identify drug quality and purity. The identification, isolation and purification of active chemical constituents is depends chemical methods of evaluation. Preliminary phytochemical investigation is also a part of chemical evaluation.

i) Phytochemical Test

Table 1

Sr. No	Phytochemical Test	Test Extract Procedure	Observation
1	Test for Flavonoids	1. Take 1-2 ml of the plant extract in a test tube. 2. Add a few drops of concentrated sulfuric acid (H_2SO_4). 3. Observe the color change.	Yellow/orange color
2	Test for Triterpenoids	1. Take 1-2 ml of the plant extract in a test tube. 2. Add 2 ml of chloroform. 3. Add 1-2 ml of concentrated H_2SO_4 along the sides. 4. Observe the layer.	reddish-brown layer
3	Test for alkaloids	1. Take 1-2 ml of the plant extract in a test tube. 2. Add 1 ml of Dragendorff's reagent. 3. Observe the precipitate formation	orange/reddish-brown precipitate
4	Test for steroids	1. Take 1-2 ml of the plant extract in a test tube. 2. Add 2 ml of chloroform. 3. Add 2 ml of concentrated H_2SO_4 along the sides. 4. Observe the ring.	reddish-brown ring
5	Test for tannins phenols	1. Take 1-2 ml of the plant extract in a test tube. 2. Add a few drops of 5% ferric chloride ($FeCl_3$) solution. 3. Observe the color change.	blue-black/greenish color

ii) Thin layer chromatography (Stahl, 2005)

Separating non-volatile mixtures is generally accomplished using thin layer chromatography (TLC). It is carried out on a sheet covered with silica gel, aluminum oxide, or cellulose, adsorbent materials. In the pharmaceutical sector especially in reference standard analyses, stability studies, and medication purity testing, TLC is very vital. On completion of the separation, each component appears as spots separated vertically. Each spot has a retention factor (R_f) expressed as:

R_f = dist. travelled by sample / dist. travelled by solvent

The factors affecting retardation factor are the solvent system, amount of material spotted, absorbent and temperature.

Steps involved in performing TLC of extracts

- **Preparation of TLC plate:** Prepared the slurry of adsorbent media (silica gel-G) in distilled water and poured the slurry on the TLC glass plates to obtain a thin layer.
- **Activation of TLC plate:** TLC plate was activated by heating in oven for 30min at 105^{O}C.
- **Sample application:** Dipping the capillary into the solution to be examined and applied the sample by capillary touched to the thin layer plate at a point about 2cm from the bottom. Air-dried the spot.
- **Chamber saturation:** The glass chamber for TLC should be saturated with mobile phase. Mobile phase was poured into the chamber and capped with lid. Allowed saturating about 30 min
- **Chromatogram development:** After the saturation of chamber and spotting of samples on plate, it was kept in chamber. The solvent level in the bottom of the chamber must not be above the spot that was applied to the plate, as the spotted material will dissolve in the pool of solvent instead of undergoing chromatography. Allowed the solvent to run around 10-15cm on the silica plate
- **Visualization:** Plates were removed and were examined visually, under UV and suitable visualizing agent (Vanillin-H_2SO_4, Methanolic $FeCl_3$ solution) after that R_f was calculated by formula.

iii) **Column Chromatography of Active Extract:**

Techniques of Isolation and Purification of Bioactive Molecules from Plants:

Modern methods have revolutionized the purification and isolation of bioactive chemicals from plants therefore allowing exact separation and identification. Using effective screening techniques, one aims to identify bioactive chemicals having cytotoxic, antibacterial, or antioxidant qualities. Cost and ethical considerations make in vitro tests favored over in vivo. Difficulties emerge from chemical complexity and plant variety. Together with ethno-botanical research, selection and gathering of plant materials help to find possible bioactive compounds. Purification is improved by solvent extraction, column chromatography, and HPLC; spectroscopic methods (UV-Vis, IR, NMR, MS) verify chemical identification.

❖ **Column Chromatography of Active Extracts:**

In preliminary phytochemical test of Gymnema Sylvestre extract, ethanol and chloroform extract show presence of different compounds. Also the TLC of both extract of shows presence of different phytometabolites.

Procedure:

1. Slurry of adsorbent (the activated silica for column) was prepared by mixing it with the solvent (mobile phase) and poured the mixture into the glass tube which contained the column solvent.

2. The cotton served to give a flat base to the column of adsorbent, which was placed into the tube before pouring the slurry.

3. Adsorbent was allowed to settle after some time sample was loaded.

4. The test sample was prepared by mixing the extract with silica powder until it became free flowing.

5. Cotton was placed above the sample loaded to avoid disturbances to sample as fresh mobile phase was added to the column.
6. During fractionation of column the level of the solvent was never allowed to fall below the level of the test sample loaded.
7. Then the column was eluted with mobile phase in isocratic manner.
8. Fractions of desired volume were collected and dried at room temperature to obtain a more concentrated fraction

Experimental column chromatography:

Height of column : 40 cm.
Diameter of column : 3.5 cm.
Stationary phase : Silica gel for column chromatography (60-120#)
Mobile phase : 1. n-Hexane,
2. Ethyl acetate
3. Methanol
Flow rate : 6-8 drops per minute.
Number of fractions Collected: 5
Volume of each fraction : 200 ml

Pharmacological Screening of Isolated Fractions

After collection of all fractions of ethanol extract, these fractions then screened for antidiabetic activity.

TLC- Characterization of Bioactive Fractions:

After pharmacological evaluation of ethanol fractions the only active fraction was evaluated by thin layer chromatography for determination of phytocomponents by following ways.

Table 2 TLC studies of plant extracts.

Sr. No	Extracts	Solvent system (v:v:v)	No. of spots
1	Gymnema Sylvestre	n-hexane: ethyl acetate (2:4)	03

Rf values have been determined and visualized through the use of many detective agents such as Dragendorff's, ninhydrin, iodine vapours, concentrated sulphuric acid and ferric chloride for selected extract following elution.

STRUCTURAL ELUCIDATION:

The above active isolated fractions of TLC spots were further characterized by and FTIR, and 1HNMR.

3. Results and Discussion

- **Standardization of plants materials:**

1. Determination of foreign organic matter:

Foreign organic matter in Whole plant extract of Gymnema Sylvestre extract powder was found when observed under 6X lens is given below in the table.

Table.3 Foreign Organic Matter

Name of Plant Extract	Foreign Organic Matter (%w/w)
Gymnema Sylvestre	0.4

It was observed that, the percentage of foreign organic matter was found to be within the limit. The limit for percentage of foreign organic matter is not more than 2%.

2. Determination of moisture content:

Moisture content was measured and it was observed that, the results were complying as per standard guidelines.

Table.4 Observation of Loss on Drying.

Name of Plant extract	Loss of moisture (%w/w) / Time (hrs.)				
	0	01	02	03	04
Gymnema Sylvestre	0.00	0.17	0.21	0.22	0.22

It was observed that the LOD for all plant extract was found to be within limit.

3. Determination of Ash value: -

Ash value analysis helps determine sample authenticity and purity, serving as an important qualitative standard. The total ash, acid-insoluble ash, and water-soluble ash values indicate the root's suitability for drug action. The water-soluble extractive value, lower than the alcohol-soluble extractive value, suggests better solubility of constituents in alcohol, highlighting its effectiveness for extraction.

Table.5 Ash value of plant extracts.

Sr. No.	Evaluation Parameters	Value (%w/w)
		Gymnema Sylvestre extract
1.	Total ash value	2
2.	Acid insoluble ash value	0.7
3.	Water soluble ash value	0.3

4. Determination of Extractive values: -
Ethanol-soluble extractive value was found to be greater than other extractive value; it indicates that compounds present in the whole plant extracts are soluble in alcohol in high amount. This might guide us for the isolation of maximum active components form plant.

Table.6 Extractive Values (%w/w)

Sr. No.	Evaluation Parameters	Extractive Values (%w/w)
		Gymnema Sylvestre extract
1.	Ethanol soluble extractive values	12.7
2.	Water soluble extractive values	6.8

❖ **EXTRACTION**

The extraction was carried out in several batches. The whole plant extract, extracted with various solvents. The appearance and percent yield of extracts are reported in below table.

Table.7 Yield of various extracts obtained from the whole plant powder. (Gymnema Sylvestre dry extract)

Sr. No.	Evaluation Parameters	Color	Nature	Percentage Yield (% W/W)
1.	Petroleum ether	Light Yellow	Oily	7.76
2.	Ethanol	Dark Green	Sticky/Semi-Solid	12.02
3.	Chloroform	Greenish-Brown,	Semi-Solid	8.41
4.	Ethyl acetate	Brownish-Yellow	Thick Liquid/Semi-Solid	6.73
5.	Aqueous	Brown,	Viscous Liquid	8.22

Preliminary Phytochemical Screening: -

Extracts were tested for the presence of active principles such as Alkaloids, Triterpenoids, Glycosides, Saponins, Tannins phenols, Flavonoids and Steroids, etc.

Table.8 Phytochemical test of Gymnema Sylvestre Extract

Sr. No.	Phytochemical test	Test Extracts
1.	**Test for Flavonoids**	
a	H2SO4 Test	+
2.	**Test for Triterpenoids**	
	Choloroform Test	+
3	**Test for alkaloids**	
	Dragondroff's reagent	+
4	**Test for steroids**	
	Salkowski Test	+
5	**Test for Glycosides**	
	FeCl3 Test	-

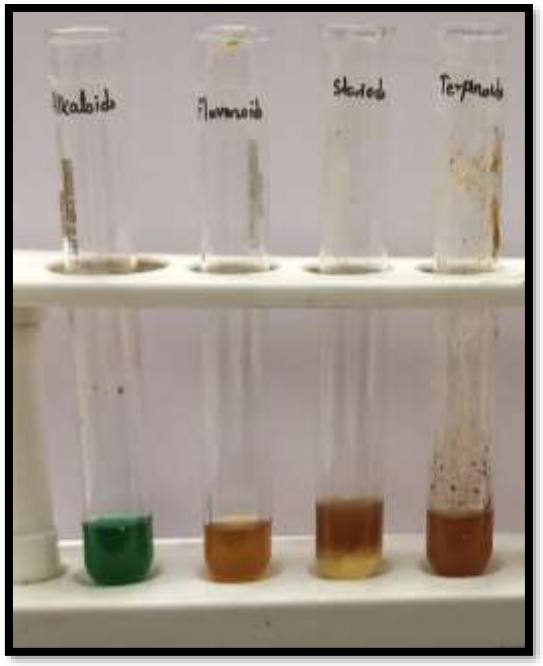

Figure.1 Phytochemical test of Gymnema Sylvestre Extract

The result of phytochemical study on Whole plant extract of Gymnema Sylvestre extract (95%) powder showed presence of flavonoids, triterpenoids, alkaloids, steroids.

Thin layer chromatography of extract

TLC chromatographic study were carried out to separate the active constituents which may having pharmacological active constituents.

Table.9 Thin Layer Chromatography of all extracts.

Sr. no.	Chemical constituent	Mobile Phase	Visualization Spraying reagent	Color of spot	Rf- value
1.	**Alkaloids**	Toluene : Ethyl acetate: Formic acid (50:40:10)	10% H_2SO_4 in ethanol	Violet - blue	Petroleum ether :0.72 Ethanol: 0.86 Chloroform : 0.85
2.	**Flavonoid**	Toluene : Ethyl acetate : Glacial acetic acid : Water (100:11:11:26)	Anisaldehyde – Sulfuric acid.	Yellowish green	Petroleum ether: 0.71 Ethanol : 0.82 Chloroform : 0.89
3.	**Triterpenoids**	Ethyl acetate: Formic acid : Acetic acid : Water (100:11:11:26)	5 % $FeCl_3$ in 0.1N HCl	Black	Ethanol : 0.65
4.	**Steroids**	Ethyl acetate : Methanol : Acetic acid (70 : 20 : 10)	Vanillin – Sulfuric acid.	Pink	Petroleum ether: 0.86 Ethanol : 0.76 Chloroform : 0.48 Ethyl acetate : 0.81

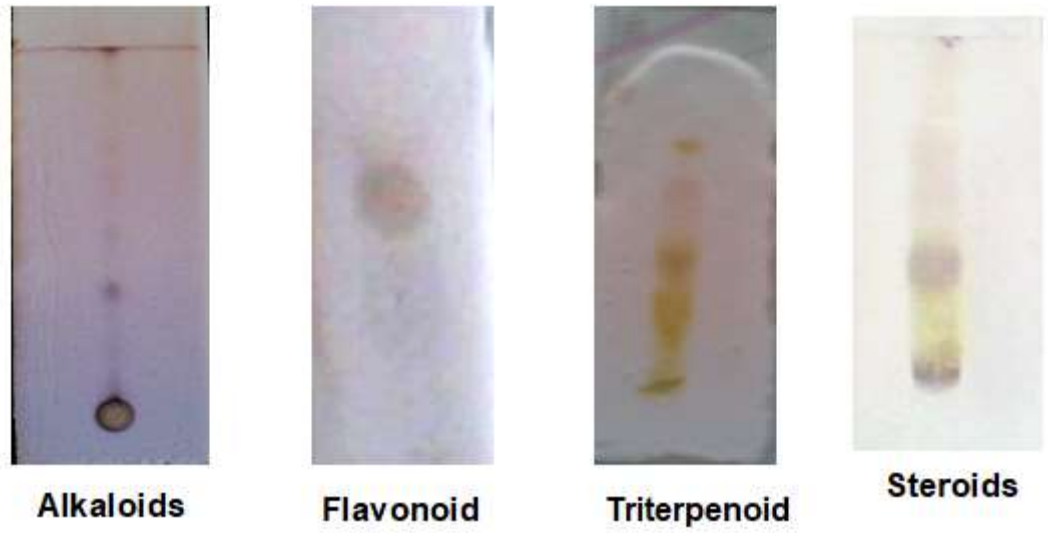

Figure.2 TLC chromatography for Alkaloid, flavonoid, Triterpenoids and steroids

- **Column Chromatography of Active Extract of All Whole Plant Extract**

In preliminary phytochemical test of whole plant of ethanol and chloroform extract show presence of Alkaloid, Steroids, flavonoids and terpenoids. Also the TLC of all extract of shows presence of different phytometabolites. The ethanol and chloroform extract shows significant potential significant Phytoconstituents. So phytocomponents from ethanol were needed to isolate using column chromatography.

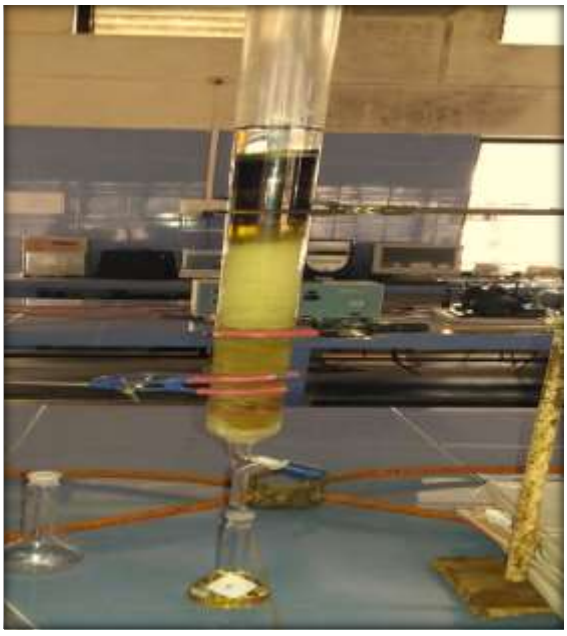

Figure.3 Column chromatography

A] Ethanol Extract Column Chromatography

Table.10 Appearance and percent yield of all fractions.

Plant Name	Mobile phase	Fraction Designation	Weight of fraction (gm.)	% Yield w/w
Gymnema Sylvestre Extract (10%)	n-Hexane: Ethyl acetate (5: 5)	GSF1	0.73	4.6
		GSF2	0.77	3.8
		GSF3	0.82	4.4

Figure.4 TLC chromatograph of Gymnema Sylvestre Extract

Table.11 RF values of plant extracts.

Sr. No	Extracts	R_f values
1	**Gymnema Sylvestre**	0.33, 0.48,0.57

Table.12 Spectroscopic data of isolated Compound-I.

Spectroscopic Techniques	Data Interpreted
IR (cm^{-1}) Kbr	3647.07 (O-H stretching), 2922.59 (C-H stretching), 3440.39 (HC=CH stretching), 1637.27(C=C), 1448.28(CH from CH2), 1371.25 [C-H stretching (CH2 (CH3)2)], 1138.76(OH-bending) 1087.66 (C-C stretch).
^{1}HNMR (DMSO)	δ 7.324-7.565 (m, 10H), δ 6.788 (s, 1H), δ 3.826-3.839 (s, 2H), δ 2.50 (s OH), δ1.256 (s, 3H), δ 1.366 (s, 2H), δ 1.014 (s, 2H)

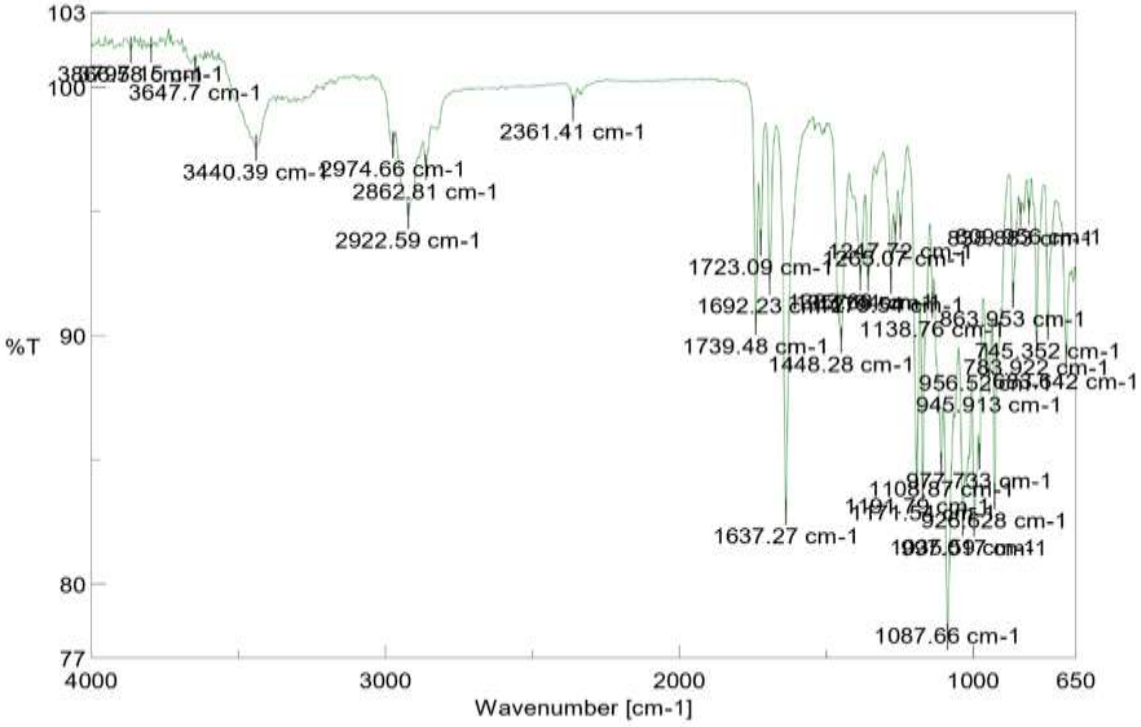

Figure.5 IR spectra of Gymnema Sylvestre

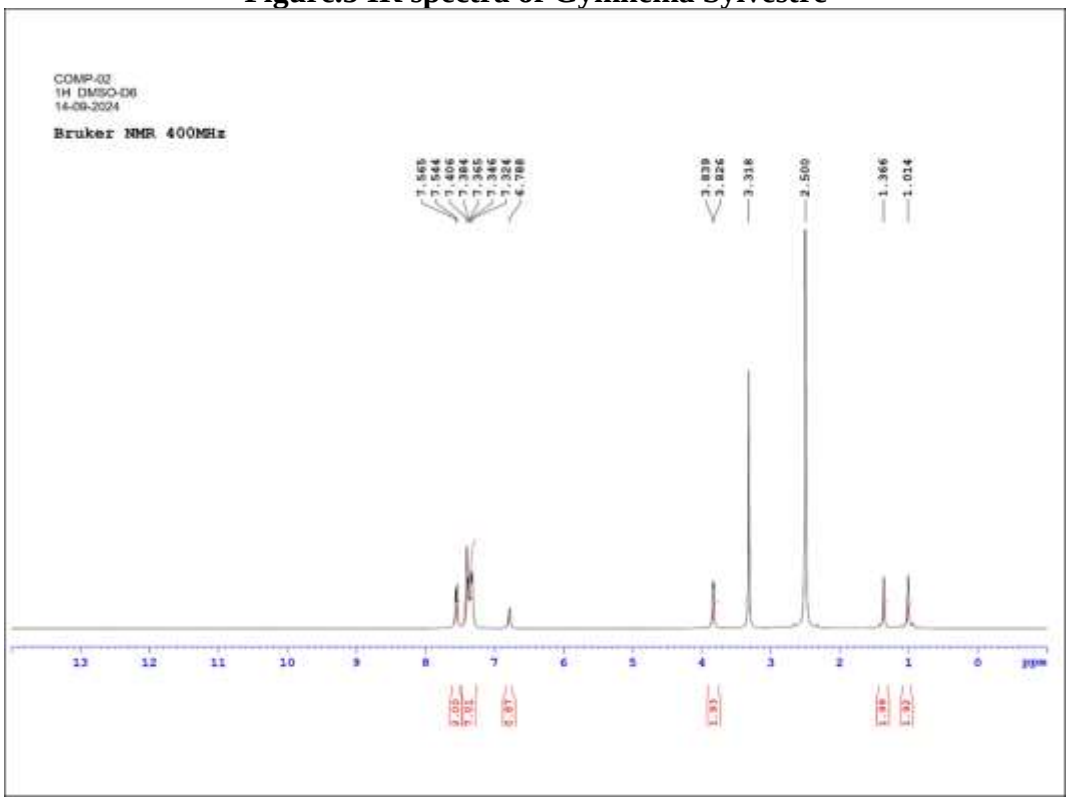

Figure.6 Proton NMR spectrum of Gymnema Sylvestre

4. Conclusion

First investigation of Gymnema Sylvestre extract-based nanoneutraceuticals revealed promising phytochemical and physicochemical properties. Standardizing studies confirmed the authenticity and purity of the plant material by means of foreign organic matter, ash values, and extractive values within tolerable limitations. Having the greatest yield, the ethanol extract showed its suit for extracting bioactive compounds.

Phytochemical screening revealed the presence of flavonoids, alkaloids, triterpenoids, and steroids confirmed by TLC analysis. Column chromatography helped to extract active fractions; spectroscopic study provided structural insight into the bioactive compounds. These findings suggest that Gymnema Sylvestre extract has probable utility in nanoneutraceutical formulations and presents bioactive components with considerable pharmacological value.

Acknowledgments

Conflict of interest:

The author declares no conflict of interest.

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