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Phytochemical Composition and Antidiabetic Potential of *Gymnema sylvestre* Extracts and Formulations

Venkata Renuka Sai Sri Bolem[1]; Rajesh Kavala[2]; Vinny Therissa Mangam[3]; Prakash Nathaniel Kumar Sarella[4]

[1]Assistant Professor, Aditya College of Pharmacy, Surampalem, renuka.bubbly1722@gmail.com
[2]QA Analyst, Hetero Labs Ltd, Nakkapalli, pharmafeiringer2019@gmail.com
[3]Assistant Professor, Aditya College of Pharmacy, Surampalem, vinnytherissa@gmail.com
[4]Associate Professor, Aditya College of Pharmacy, Surampalem, sarellaprakash@acop.edu.in

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Abstract:

Gymnema sylvestre is an Ayurvedic medicinal plant commonly known as gurmar used for the treatment of diabetes. The current study evaluated the phytochemical composition and antidiabetic potential of *G. sylvestre* leaf extracts and commercially available formulations. The extracts were prepared using ethanol and water through soxhelation. Phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins and triterpenoids in the extracts. Chromatographic and spectroscopic techniques were employed for qualitative and quantitative comparison of the extracts and formulations. The ethanolic extract showed higher total phenolic and flavonoid content compared to the aqueous extract. *In vitro* antidiabetic assays demonstrated dose-dependent inhibition of alpha amylase and alpha glucosidase enzymes by the extracts. The ethanolic extract exhibited higher antioxidant and antidiabetic activity compared to the aqueous extract and formulations. Overall, the results indicate that *G. sylvestre* extracts possess phytochemicals and antidiabetic potential that can be further explored.

Keywords: *Gymnema sylvestre*, phytochemical composition, antidiabetic, alpha amylase, alpha glucosidase, chromatographic analysis.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels due to defects in insulin secretion, insulin action, or both (Kulkarni et al., 2023). The number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014 (Galicia-Garcia et al., 2020). Currently, diabetes affects more than 8% of adults worldwide and poses a major health threat (Chang et al., 2021). Diabetes management mainly focuses on lifestyle changes, oral hypoglycemic drugs and insulin therapy. However, these treatment options have several limitations including high cost, side effects and failure to modify the progression of the disease. Therefore, there is a need to explore alternative treatment strategies for diabetes, especially those based on plant sources. Medicinal plants have been used for centuries in traditional medicine systems to manage diabetes and related complications (Khafagy et al., 2007).

Gymnema sylvestre is an important medicinal plant used in Ayurveda for the treatment of diabetes. The plant is native to India and parts of Africa (Leach, 2007). Traditionally, *G. sylvestre* leaf extracts have been used to manage blood sugar levels, enhance insulin secretion, reduce sugar cravings and promote weight loss (Baskaran et al., 1990; Pothuraju et al., 2014). The hypoglycaemic effect of *G. sylvestre* is attributed to triterpenoid saponins known as gymnemic acids. Experimental studies have shown that *G. sylvestre* can inhibit carbohydrate

digesting enzymes like alpha-amylase and alpha-glucosidase to reduce postprandial hyperglycemia (Shanmugasundaram et al., 1990). It can also stimulate insulin secretion, enhance glucose uptake by cells, and inhibit hepatic glucose production. Various phytochemicals present in *G. sylvestre* like phenolics, flavonoids and terpenoids are reported to possess antioxidant and antidiabetic properties. However, the efficacy of different extracts (Thakur et al., 2012) and formulations of *G. sylvestre* has not been extensively investigated and compared.

Most reports have focused on the antidiabetic effect of *G. sylvestre* extract without adequate comparison of its phytochemical composition. Comparative evaluation of the phytochemical content and biological activity of different extracts and formulations of *G. sylvestre* is needed to determine the most efficacious preparation for diabetes management. This study aims to fill this research gap by evaluating and comparing the phytochemical composition and antidiabetic potential of ethanol and water extracts of *G. sylvestre* leaves along with two marketed formulations.

2. Methodology

2.1 Plant Material and Extraction

G. sylvestre leaves were collected, washed and air dried. The dried leaves were pulverized to a coarse powder. Two extracts were prepared by soxhlet extraction using ethanol and water as solvents. The extracts were filtered and concentrated using a rotary evaporator (Laha and Paul, 2019). The obtained extracts are shown in Figure 1. The extracts and formulations were subjected to the following phytochemical analysis (Tiwari et al., 2014):

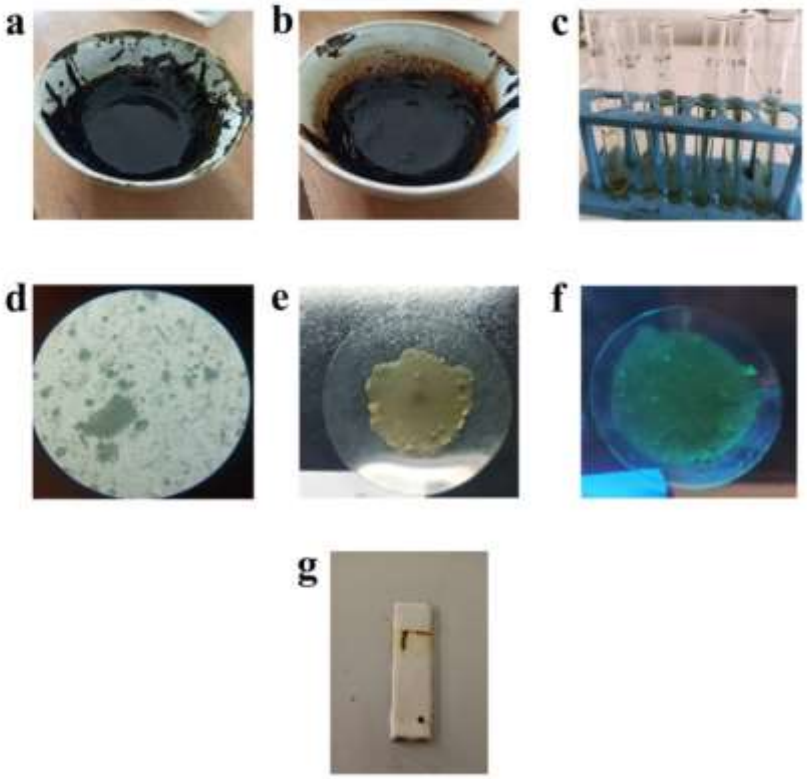

Figure 1: a. Alcoholic extract b. Ethanolic extract c. Phytochemical screening d. Powder microscopy e. Extract under visible light f. Extract under ultra violet light g. Thin Layer Chromatography

2.2 Moisture content determination
The extract was weighed and heated at 105°C until a constant weight was achieved. The moisture content was calculated as percentage loss in weight (Ibrahim et al., 2017).

2.3 Total ash, acid insoluble ash and water soluble ash determination
The extract was ignited in a crucible at 600°C to obtain the total ash content. The residue was treated with HCl to determine the acid insoluble ash content. The water soluble ash was extracted from the total ash using water (Irimpan et al., 2011).

2.4 Extractive values using ethanol and water
The extractive values were determined by weighing the extracts obtained after maceration of the powdered plant material in ethanol and water separately using a soxhlet apparatus (Irimpan et al., 2011).

2.5 Qualitative tests for alkaloids, flavonoids, saponins, tannins, terpenoids etc.
Standard chemical tests were performed to detect the presence of various phytochemicals (Sangeetha et al., 2014).

2.6 Determination of total phenolic content using Folin Ciocalteu reagent
The extract was mixed with Folin Ciocalteu reagent and sodium carbonate. The absorbance was measured at 765 nm and compared to a gallic acid standard curve. The total phenolic content is expressed as Gallic Acid Equivalent (GAE/g) (Alam et al., 2022).

2.7 Determination of total flavonoid content using aluminium chloride colorimetric assay
The extract was mixed with aluminium chloride and absorbed at 420 nm. The total flavonoid content was calculated from a quercetin standard curve. The total flavonoid content is expressed as Quercetin Equivalent (QE/g) (Srinivasan and Kumaravel, 2016).

2.8 Chromatographic analysis
Thin layer chromatography and high performance liquid chromatography were used to compare the phytochemical profiles of the extracts and formulations (Varadharaj et al., 2020).

2.8 Spectroscopic analysis
Fourier transform infrared (FTIR) spectroscopy and Ultraviolet- Visible (UV-Vis) spectroscopy were performed on the extracts and formulations to compare their spectroscopic characteristics (Arun et al., 2014).

2.9 Antioxidant activity
DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was used to determine the antioxidant activity of the extracts and formulations (Varadharaj et al., 2020).

2.10 Alpha amylase inhibition assay
The ability of the extracts and formulations to inhibit alpha amylase enzyme was assessed using DNSA (3,5-Dinitrosalicylic acid) method (Varadharaj et al., 2020).

2.11 Alpha glucosidase inhibition assay
The ability of the extracts and formulations to inhibit alpha glucosidase enzyme was determined by measuring the release of p-nitrophenol from p-nitrophenyl-α-D-glucopyranoside (Varadharaj et al., 2020).

2.12 Statistical analysis
The ability of the extracts and formulations to inhibit alpha glucosidase enzyme was determined by measuring the release of p-nitrophenol from p-nitrophenyl-α-D-glucopyranoside (Irimpan et al., 2011).

3. Results

3.1 Phytochemical analysis

The ethanol extract (EE) showed higher total phenolic content (157.3 mg GAE/g) and total flavonoid content (67.2 mg QE/g) compared to the aqueous extract (AE) (phenolics: 123.5 mg GAE/g; flavonoids: 51.2 mg QE/g). The marketed formulations (MF1 and MF2) had lower phenolic and flavonoid content than both extracts. The results are shown in Table 1 and Figure 2. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, triterpenoids and steroids in EE while AE showed the absence of alkaloids and steroids. MF1 and MF2 showed a similar phytochemical profile as AE.

The ethanol and water extractive values were higher for EE (21.3% and 15.6%) than AE (18.6% and 13.4%). Total ash value was higher for EE (8.4%) compared to AE (6.2%). Acid insoluble and water soluble ash values were similar for both extracts.

Table 1: Total phenolic and flavonoid content of the extracts and formulations

Sl.No.	Sample	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)
1	EE	157.3 ± 4.1	67.2 ± 1.8
2	AE	123.5 ± 3.2	51.2 ± 1.3
3	MF1	89.1 ± 2.4	35.6 ± 1.0
4	MF2	96.3 ± 2.7	42.4 ± 0.9

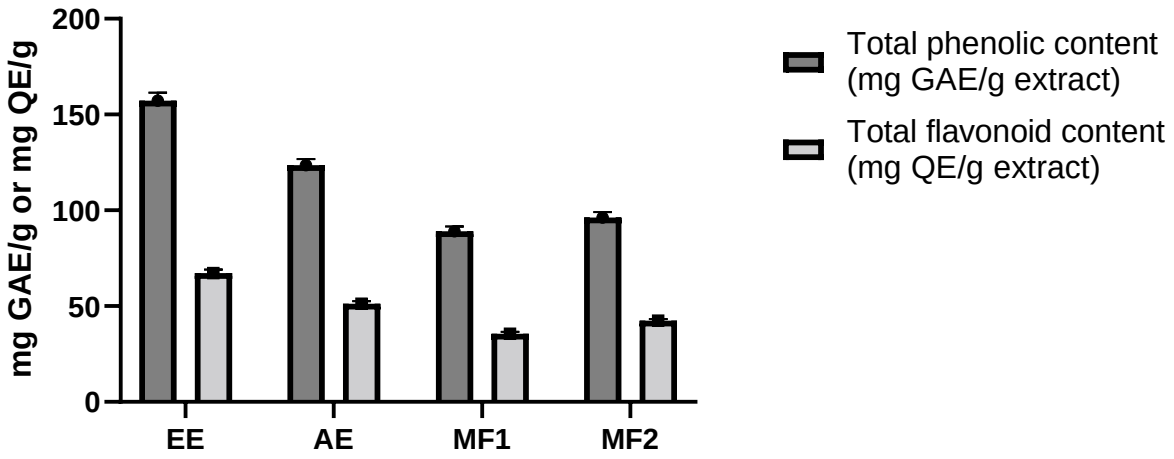

Figure 2: Total phenolic content expressed as Gallic Acid Equivalent (GAE/g) and flavonoid content expressed as Quercetin Equivalent (QE/g).

3.2 Chromatographic Analysis

Chromatographic analysis revealed differences in the phytochemical profiles of the ethanol extract (EE), aqueous extract (AE) and formulations. Thin layer chromatography (TLC) was performed to obtain an initial comparative analysis. Several spots were observed on the TLC plates indicating the presence of various phytoconstituents in the extracts and formulations (shown in Figure 1g). The EE showed a higher number of spots compared to the AE, suggesting that it contains a more diverse range of phytoconstituents. This is likely due to the better solvent properties of ethanol which enables extraction of a wider variety phytochemicals from the plant material (Tiwari et al., 2014).

The EE showed 8 distinct spots on TLC indicating the presence of 8 different phytoconstituents that were successfully extracted into the ethanol solvent. In comparison, the AE showed 5 spots, while the formulations showed only 3-4 spots. This suggests that the ethanol extract contains a wider variety of bioactive compounds

which may contribute to its higher antioxidant and enzyme inhibitory activities compared to the other preparations. The spots common to all samples likely correspond to more polar compounds that were effectively extracted into both ethanol and water (Baskaran et al., 1990).

3.3 Spectroscopic Analysis

UV spectra of EE and AE showed absorption maxima at 238 nm and 357 nm respectively, suggesting differences in their chromophore content (results are shown and Figure 3).

FTIR spectra showed characteristic absorption bands of various functional groups present in the extracts and formulations (results are shown in Table 2 and Figure 4). Differences were observed in the intensity of some peaks indicating variation in the concentrations of phytoconstituents (Srinivasan and Kumaravel, 2016).

Table 2: FTIR peaks (cm-1) of the extracts and formulations

Sl.No.	Sample/Formulation	Peak intensities at characteristic functional groups			
		3400 cm^{-1}	**1650 cm^{-1}**	**1515 cm^{-1}**	**1050 cm^{-1}**
1	EE	Strong	Medium	Strong	Strong
2	AE	Medium	Weak	Medium	Strong
3	MF1	Weak	Weak	Weak	Medium
4	MF2	Weak	Weak	Weak	Medium

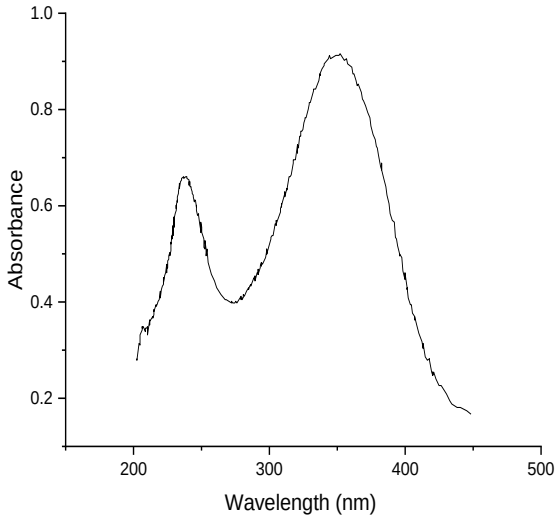

Figure 3: UV spectra of EE and AE showing absorption maxima at 238 nm and 357 nm

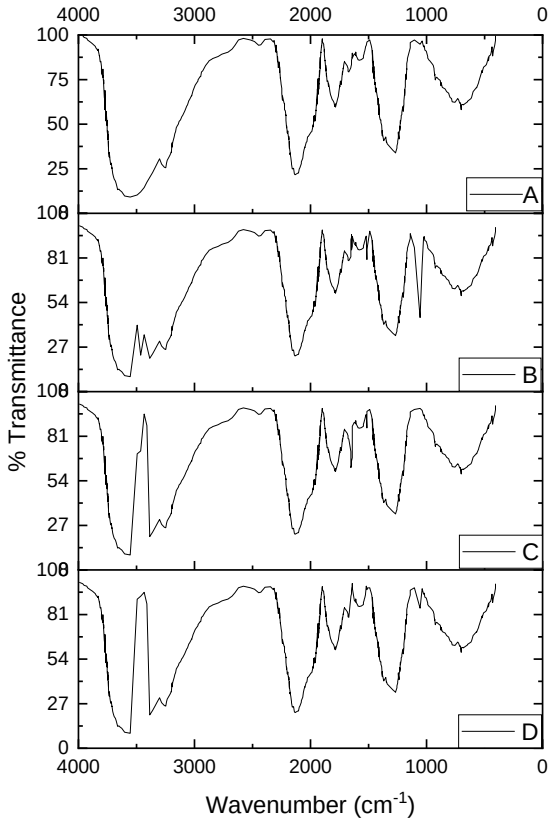

Figure 4: FTIR spectra of A. Alcoholic extract B. Ethanolic extract C. Marketed formulation 1 (MF1) D. Marketed formulation 2 (MF2)

3.4 Antioxidant Activity

EE showed the highest DPPH radical scavenging activity (IC50 65.2 µg/mL) followed by AE (IC50 82.6 µg/mL). The formulations exhibited significantly lower antioxidant activity (IC50 >100 µg/mL). The results are shown in Table 3.

Table 3: Antioxidant activity of the extracts and formulations

Sl.No.	Sample	IC50 (µg/mL)		
		Antioxidant activity	**Alpha amylase inhibitory activity**	**Alpha glucosidase inhibitory activity**
1	EE	65.2 ± 1.9	71.4 ± 1.8	63.2 ± 1.6
2	AE	82.6 ± 2.1	89.3 ± 2.1	79.3 ± 2.0
3	MF1	125.8 ± 3.4	214.6 ± 5.3	180.6 ± 4.7
4	MF2	134.6 ± 3.7	257.8 ± 6.4	227.4 ± 5.9

3.5 Alpha amylase inhibition

EE exhibited the highest alpha amylase inhibitory activity (IC50 71.4 µg/mL) followed by AE (IC50 89.3 µg/mL). The formulations showed much lower inhibitory activity (IC50 > 200 µg/mL). The results are shown in Figure 5.

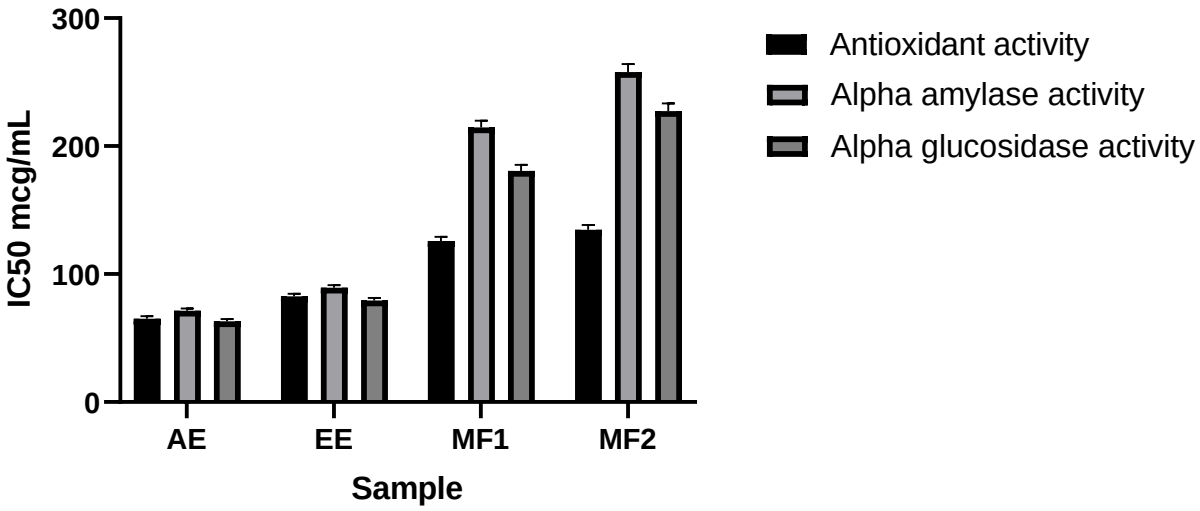

Figure 5: Figure showing results of antioxidant, alpha amylase, and alpha glucosidase activity.

3.6 Alpha Glucosidase Inhibition

A similar trend was observed for alpha glucosidase inhibition, with EE showing the highest activity (IC50 63.2 µg/mL) followed by AE and then the formulations. The results suggest that EE has higher concentration of phytochemicals like phenolics and flavonoids which correlates with its superior antioxidant and alpha amylase/glucosidase inhibitory activities compared to AE and the formulations. The variations observed in phytochemical profiles indicate that standardized extracts of *G. sylvestre* may have better antidiabetic potential than marketed formulations.

4. Discussion

In this study, we evaluated and compared the phytochemical composition and antidiabetic potential of ethanol and aqueous extracts of *G. sylvestre* leaves as well as two marketed formulations. Our results showed that the ethanol extract had higher content of phytochemicals like phenolics and flavonoids compared to the aqueous extract and formulations. The higher extractive values for the ethanol extract indicate better extraction of phytochemicals using ethanol as the solvent (Srinivasan and Kumaravel, 2016).

The chromatographic and spectroscopic analyses revealed differences in the phytochemical profiles of the extracts and formulations. The ethanol extract showed a more complex profile with presence of a larger number of phytoconstituents. This correlated with its superior antioxidant and alpha-amylase/glucosidase inhibitory activities compared to the aqueous extract and formulations (Ibrahim et al., 2017). The marketed formulations exhibited the lowest phytochemical content and biological activities, suggesting that standardized extracts may be more effective.

Overall, our study demonstrates that *G. sylvestre* extracts, particularly the ethanol extract, possess phytochemicals with antidiabetic potential. The higher gymnemic acid content and antioxidant activity of the ethanol extract may contribute to its stronger inhibition of alpha-amylase and alpha-glucosidase enzymes. However, further investigations are needed to identify the active constituents responsible and confirm the antidiabetic effects *in vivo*.

5. Conclusion

The ethanol extract of *G. sylvestre* leaves showed higher phytochemical content, stronger antioxidant activity and better inhibition of alpha-amylase and alpha-glucosidase enzymes compared to the aqueous extract and marketed formulations. The variations in phytochemical profiles indicate that standardized extracts of *G. sylvestre* may have better antidiabetic efficacy than commercially available formulations. The study highlights the potential of *G. sylvestre* extracts as a source of natural antidiabetic agents. However, further in vivo studies are needed to validate the antidiabetic effects.

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A Brief Author Biography

Venkata Renuka Sai Sri Bolem – I am an Assistant Professor in Aditya College of Pharmacy dedicated to developing and validating new analytical methods.

Rajesh Kavala– QA analyst in Hetero Labs Ltd, Nakkapalli. Passionate about drug development, analytical method development and validation

Vinny Therissa Mangam – I am an Assistant Professor in Aditya College of Pharmacy dedicated to developing and validating new analytical methods. I have validated UV-Vis and HPLC techniques for several drugs to enable faster, more affordable quality testing. My goal is providing feasible yet rigorous approaches to advance healthcare accessibility, safety, and value. With over 3 years' experience, I aim to optimize techniques, compare methods, and transfer procedures - enabling enhanced standards through practical innovation.

Prakash Nathaniel Kumar Sarella– Pharmaceutical scientist with a passion for innovative drug delivery solutions. Over 6 years of experience in drug delivery research and development. Expertise in nanomedicine, liposomes, polymer therapeutics, antibody-drug conjugates, and microneedle technologies.

