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PHARMACEUTICAL COMPOSITION FOR MANAGING DIABETES WITH BERBERINE AND GYMNEMA SYLVESTRE EXTRACTS

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ABSTRACT

The Present study discloses a composition for Managing Diabetes with *Berberine* and *Gymnema Sylvestre* Extracts. The Diabetes mellitus is a challenging human disorder for medical researchers, and the patients suffering from it are from all age groups. These Plants are recognized to play a crucial role as protective agent against diabetes mellitus major parts of the plant flowers leaves have been used as conventional drugs since hundreds of years. The phytochemical analysis of the methanolic extracts of *Berberine* and *Gymnema Sylvestre* extracts in-house and marketed samples showed presence of flavonoids, steroids, alkaloids, terpenoids, phenols, saponins and tannins showed a ability to maintain β-cells performance and decrease glucose levels in the blood further *In-vitro* study was performed by using the standard protocol against plant extract. The *In-vitro* antidiabetic activity was performed by the alpha-amylase inhibition method. Inhibition of the alpha amylase enzyme would delay the degradation of carbohydrates, which would in turn cause a decrease in the absorption of glucose and a reduction in postprandial blood glucose levels. Based upon the experimental evidences, it was found that composition of *Berberine* and *Gymnema sylvestre* possesses higher anti-diabetic activity.

Keywords: *Berberine*, *Gymnema Sylvestre,* Diabetes, *In-vitro* α-amylase

INTRODUCTION

Diabetes mellitus (DM) is a major public health problem and one of the most challenging diseases worldwide. According to the World Health Organization (WHO), second highest rate of diabetes in the Middle east and seventh highest globally. Some diabetic patients may prefer to use alternative approaches such as herbal remedies to control their blood glucose level and this study aims to use *Berberine* and *Gymnema Sylvestre* Extracts to manage diabetes.

Berberine is an isoquinoline quaternary alkaloid (or a 5, 6 dihydrodibenzo[a,g]quinolizinium derivative) isolated from many medicinal plants such as *Hydrastis canadensis, Berberis aristata, Coptis chinensis, Coptis rhizome, Coptis japonica, Phellondendron amurense and Phellondendron* chinense schneid **[1-4]**. *Berberine* is traditionally used for its supposed antimicrobial effects and as treatment for diabetes in traditional Chinese, Indian and Middle Eastern folk medicine [**2-8]** and has definite potential as a drug included in a wide spectrum of clinical applications. *G. sylvestre* is a medicinal plant belonging to the Asclepiadacea family popularly known as "gurmar" in Hindi, which means "sugar destroying" **[9-12]**. It is a woody climber that grows in tropical forests in India and South East Asia. Its leaves exhibit a broad range of therapeutic effects due to its active ingredients referred to as gymnemic acids **[13. 14]**.

MATERIAL AND METHOD

1. Plant Extract Preparation

The plants were further identified and authenticated each sample. The dried leaves were coarsely powdered using a mortar. Then, this coarsely powdered plant was macerated in 80% methanol to obtain the hydro-alcoholic crude extract using Erlenmeyer flask for 3 days at room After 72 h, the filtrate was separated from the marc by using filter paper (Whatman No. 1).and the crude extracts were obtained after reduced pressure concentration.

2. Phytochemical analysis

The phytochemical analysis of the extracts and fractions was carried out by following methods

Alkaloids: A volume of 1cm^3 of 1% HCl was added to 3 cm^3 of the extracts in a test tube. The mixture was heated for 20 minutes, cooled and then filtered. The filtrate was used as follows.

A. Two drops of Mayers reagent were added to 1cm^3 of the extracts. A creamy precipitate indicated the presence of alkaloids in the extracts.

B. Two drops of Wagner's reagent were added to 1cm^3 of the extracts. A reddish brown precipitate indicated the presence of alkaloids

Tannins: A volume of 1cm³ of freshly prepared 10%w/v KOH was added to 1cm³ of the extracts. A dirty white precipitate indicated the presence of tannins.

Phenolics: Two drops of 5% $FeCl_3$ were added to 1cm³ of the extracts in a test tube. A greenish precipitate indicated the presence of phenolics.

Glycosides: A aliquot of 10 cm³ of 50% H_2SO_4 was added to 1cm³ of the extracts, the mixture was heated in boiling water for 15 minutes. Then, 10 cm³ of Fehling's solution was added and the mixture boiled. A brick red precipitate indicated the presence of glycosides.

Saponins: Frothing test: A volume of 2 cm³ of the extract in a test tube was vigorously shaken for 2 minutes. Frothing indicated the presence of saponins.

A. Emulsion test: Five drops of olive oil were added to 3 cm³ of the extract in a test tube and vigorously shaken. A stable emulsion formed indicated the presence of saponins.

Flavonoids: An aliquot of 1cm³ of 10% NaOH was added to 3cm³ of the extracts. A yellow colouration indicated the presence of flavonoids.

Steroids: Salkowsti test: Five drops of concentrated H_2SO_4 were added to 1cm³ of the extracts. A red colouration indicated the presence of steroids

Preparation of Thin-layer Plates

Clean TLC plates by developing in one or more polar, neutral solvents, in order to move adsorbed contaminants away from the zone of development. In a fume hood, prepare enough cleaning solvent (e.g. 15-100 ml of ethyl acetate-methanol 2:1, v/v) to cover the bottom of the TLC developing chamber, as well as the lower edge of a TLC plate when set inside the chamber. Use commercially available glass TLC developing chambers (different sizes available, with lids) or foil-covered Pyrex beakers or preserving jars. Use scissors to cut aluminum- or plastic-backed (flexible) silica gel plates, which come in various sizes (20 cm x 20 cm and smaller), to fit the available developing chamber. (Caution: silica can cause lung damage if inhaled. Work in a fume hood, and handle TLC plates with gloves to avoid getting skin oils onto the silica). Insert the plates into the chamber, with the tops leaning against the chamber walls. Plates should not touch each other. Cover the chamber and let the solvent move up the plate by capillary action. When solvent has reached the top of the plates, remove plates from chamber and arrange in a standing position within the fume hood until solvent has evaporated. Check to see if impurities have migrated near the top of the TLC plate by looking for a yellow band under visible light, or a fluorescent band under ultraviolet (UV) light (see the "impurity front" or IF in **Figure 1**). If the

majority of the plate still has a yellowish tinge, repeat the cleaning process. After removing TLC plates from the chamber, discard the solvent. Allow residual solvent to evaporate completely. To remove residual moisture that can affect migration of compounds on silica16, prop the plates upright in a drying oven at 100 °C (10-15 min for a 20 cm x 20 cm plate, and 5 min for 7 cm x 10 cm plates).If a 100 °C drying oven is not available, heat plates for a longer period of time at lower temperatures (i.e. 40 min at 60 °C).After the plates are dry, let them cool to ambient temperature before loading.

Preparation of Developing Chambers for Extract Separation

Use scissors to cut a piece of filter paper slightly below chamber height, and about half the chamber perimeter in width. This paper acts as a wick to draw solvent up the chamber wall and saturate the chamber with solvent vapours, thus improving reproducibility of separations1.In a fume hood, mix solvents (ethyl acetate-methanol 4:1, v/v, for this study). Pour solvent mixture into the chamber and cover. Wait until the entire wick is wet with solvent, indicating chamber saturation, to put plates into chamber.

Loading and Development of TLC Plates

Lightly mark the origin with pencil. If the TLC plate adsorbent is soft and easily damaged, make marks at edges. Compounds should be above the surface of the developing solvent when plates are inserted into the TLC chamber. Dissolve extracts in enough organic solvent (in this case, methanol) to have a concentrated solution instead of a turbid suspension. Load samples and standards as narrow bands with a microliter syringe or capillary micropipettes, leaving a 1 cm border on the sides of the plate. Allow the bands to dry (fanning the plate or loading it in a fume hood helps). If a greater concentration of sample is needed on a plate, "overspot" by loading samples again on the dried bands. With forceps or tongs, set plate(s) inside the saturated TLC chamber. Plates should not touch the wick because it may provide solvent to the plates at points of contact, thus altering the path of compound migration. Cover chamber and let plates develop.

***In-vitro* anti-diabetic activity**

Inhibition of the alpha-amylase enzyme stirring 0.1 g of potato starch in 100 ml of 16 mM sodium acetate buffer produced a starch solution (0.1 % w/v). Alpha-amylase in the amount of 27.5 mg was combined with 100 ml of distilled water to create the enzyme solution. The 3, 5-dinitrosalicylic acid solution and sodium potassium tartrate solution are combined to create the colorimetric reagent. The alkaline conditions of an alpha-amylase solution at 25 °C were applied to a starch solution, and both control and plant extracts were added.

Three minutes were used to measure the response. By reducing 3, 5-dinitrosalicylic acid to 3- amino-5-nitrosalicylic acid, the production of maltose was measured. As according Malik and Singh (1980), the response can be observed at 540 nm

Inhibitory concentration calculation (IC50)

The percentage scavenging activities at five different concentrations of the extract were used to determine the concentration of the plant extracts needed to scavenge 50% of the radicals (IC50). The percentage of inhibition (I%) was determined using I%=AC-AS/AC 100

RESULTS AND DISCUSSION

The Present *In-vitro* comparative studies and the Physiochemical analysis between two medicinal plants *Berberine* and *Gymnema sylvestre* was performed that help reduce blood glucose levels. In this aspect, Phytochemical screening was performed and found to have: flavonoids, alkaloids, steroids, saponins, glycosides other constituents. as shown in **Table 1**.

Table 1: Pytochemical Screening results of *Berberine* and *Gymnema sylvestre*

S. No.	Chemical test	*Berberine*	*Gymnema sylvestre*
1.	Alkaloids	+	+
2.	Tannins	-	-
3.	Phenolics	-	-
4.	Glycosides	+	+
5.	Saponins	+	+
6.	Flavonoids	-	-
7.	Steroids	+	+

The developed TLC methods resolve the *berbeine* the Rf value of nearly about 0.7 and *Gymnema sylvestre* Rf value of nearly about 0.8. confirming the presence of *berbeine* and *Gymnema sylvestre* in the plants extract visualized by yellow band as shown in **Figure 1**.

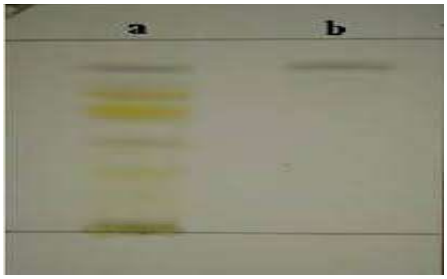

Figure 1: TLC Plate *berbeine* and *Gymnema sylvestre*

In-vitro antidiabetic activity was performed for *Berberine* and *Gymnema sylvestre* by α-amylase method. The results illustrated that *Berberine* and *Gymnema sylvestre* possesses

higher antidiabetic activity. Inhibition of the alpha amylase enzyme would delay the degradation of carbohydrates, which would in turn cause a decrease in the absorption of glucose, resulting in the reduction of postprandial blood glucose levels. Based upon the experimental evidence, it was found that *Berberine* and *Gymnema sylvestre* possesses higher antidiabetic activity. The percentage of inhibitory concentration of antidiabetic activity depends on the concentration of extracts. The **Table 2** shows the percentage of inhibitory concentrations that inhibit the alpha-amylase enzyme.

Table 2: *In-vitro* antidibetic activity result of *Berberine* and *Gymnema sylvestre* by α-amylase method

S. No.	Concentration of the Sample (ml)	Percentage of Inhibitory Concentration	
		Berberine	*Gymnema sylvestre*
1.	0.2	67	68
2.	0.4	70	72
3.	0.6	75	77
4.	0.8	80	83
5.	1.0	90	92

CONCLUSION:

The present study results, concluded that the 2 different varieties *Berberine* and *Gymnema sylveste* show similar pytochemical results that mean they contain same bioactive compounds. Based on *In-vitro* antidiabetic activity by α-amylase method. The results illustrated that *Berberine* and *Gymnema sylvestre* possesses higher antidiabetic activity.

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CONFLICTS OF INTEREST

There is no conflict of interest. I am reporting that I have not received funding from any company that may be affected by the research reported in the enclosed paper

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