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Gymnema sylvestre R. Br., an Indian Medicinal Herb: Traditional Uses, Chemical Composition, and Biological Activity

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Abstract: *Gymnema sylvestre* R. Br. is one of the most important medicinal plants that grows in tropical forests in India and South East Asia. Its active ingredients and extracts of leaves and roots are used in traditional medicine to treat various ailments and they are present in the market for pharmaceutical and parapharmaceutical products. Commercial products based on substances of plant origin that are generally connoted as natural have to be subjected to monitoring and evaluation by health authorities for their potential impacts on public health. The monitoring and evaluation of these products are critical because the boundary between a therapeutic action and a functional or healthy activity has not yet been defined in a clear and unambiguous way. Therefore, these products are considered *borderline products*, and they require careful and rigorous studies, in order to use them as complement and/or even replacement of synthetic drugs that are characterized by side effects and high economic costs. This review explores the traditional uses, chemical composition and biological activity of *G. sylvestre* extracts, providing a general framework on the most interesting extracts and what are the necessary studies for a complete definition of the range of activities.

Keywords: Antisweet activity, biological activity, functional foods, *Gymnema sylvestre*, gymnemic acid, phytochemical investigation, traditional uses.

1. INTRODUCTION

In recent years, interest in the biological activity of naturally occurring substances has grown steadily. In fact, it is well known that there has been an increase in the use of food supplements and various substances based on natural products [1-5], which are often neither standardized nor thoroughly studied with respect to their possible beneficial or adverse consequences. This invokes the need for careful and rigorous studies of natural substances, with the aim of using them to complement and/or even replace synthetic drugs that are characterized by side effects [6] and high economic costs [7]. Moreover, the progressive shortage of antimicrobial drugs coupled with the associated and growing drug resistance [8] requires new treatments that are easily prescribed, cost effective, and thus from natural sources. Therefore the full attention of researchers should be devoted to rigorous experimental studies of such substances [9].

When we discuss plant drugs, we refer to the plant or part of the plant that is used medicinally (roots, leaves, seeds, bark, etc.). These plants contain chemical compounds that are likely to have 'pharmacological action' (active ingredients), as well as substances of no or little practical interest. In the world of natural substances there are thousands of drugs that are found singly or in mixtures, which are used as complements or as alternatives to conventional therapies [10-12] or even for non-medical purposes [13, 14]. Complementary medicine includes all health care practices that are not part of the tradition of a country. Traditional medicine represents the body of knowledge, practices, methods, and beliefs of different populations, based on observations and experiences that are passed on from generation to generation and used to prevent and eliminate imbalances in the physical, mental and social well-being of individuals [15].

Plants used as a complementary or alternative treatment have been and continue to be the subject of studies for the detection of single molecules or phytocomplexes with therapeutic activities. In fact, while their traditional uses are valid, the knowledge of these plants is not sufficient for them to be used safely in modern medicine. However, through phytochemical and biological screening, we aim to determine if these plants can be used with positive, practical and safe therapeutic results. Knowledge of the therapeutic properties of many plants used in traditional medicine has been handed down since the beginning of civilization and these plants have been used as healing herbs on the basis of observations.

With the introduction of new techniques for the isolation and chemical synthesis of biologically active molecules, the scientific world has been moving towards synthetic products. This change has been driven by several factors, such as the

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difficulty in finding new plant sources, the slow processes of extraction and, last but not the least, the high cost of this approach. However, in the last decades of the twentieth century there was a resurgence in plant interest, which was appreciated by a growing number of consumers who chose to return to nature for disease prevention and general health care. Most of the world population currently prefers traditional herbal medicine [16].

Since the 1990s, the WHO has aroused interest in traditional medicine in developing countries and has stimulated studies of the medicinal plants used in these areas to give scientific value to the empirical practice so that their use can be justified for primary care [17-19].

The monitoring and evaluation of natural substances are critical because the boundary between a therapeutic action and a functional or healthy activity has not yet been defined in a clear and unambiguous way. Therefore, these products are considered *borderline products*. The categories that include products of natural origin are represented by: medicinal products, food supplements, cosmetics and medical devices. There is a great deal of interest in products of plant origin in each of the respective industries. Areas of further application development are those represented by veterinary, cosmetic and homeopathic products. Research in the field of natural substances should aim to demonstrate the effective use of direct plant products (extracts or infusions), in therapy or foodstuffs. In general, we used two types of approach to study the activity of natural active substances: to characterize the single active substance or to study a natural extract in its entirety.

In the first case, it is necessary to achieve the isolation and characterization of each single molecule, the active ingredient(s),that often prove to be less active in isolation than in the original mixture [20-22].

The alternative to this approach is to study the activity of the plant complex in its entirety.

One approach is not superior to the other, they are just different. The common aim is to identify a medicinal substance or mixture that possesses curative activity and is safe with regard to adverse events.

Many single molecules with high degrees of purity are well known to be useful in emergencies and in serious clinical situations in which there is the need for effective and fast-acting pharmacological intervention, for example, adrenaline, cardiotonics, antiarrhythmics, antihistamines, antibiotics, etc. Many of these drugs are semisynthetic derivatives of natural substances [23]. The plant world represents the most important reservoir of substances with pharmacological activity [24]. For plant-derived materials, it is important to emphasize the concept of phytocomplexes. A phytocomplex can be defined as an ensemble of individual molecules with specific activities present in a plant that is associated with other constituents that do not have clear pharmacological activities, but most likely support the active molecules. It seems that the harmonious and synergistic presence of all the substances in a phytocomplex is responsible for the therapeutic activity of the drug plant in its entirety [25].

Herbal remedies can be used to treat mental and physical fatigue, some endocrine imbalances, liver failure, constipation, high cholesterol, and venous insufficiency, to give just a few examples.

Therapeutic products on the market today are often the result of a compromise between tradition and market demand [26]. A natural source can possess a variety of therapeutic properties, which must be supported by clinical findings. In other words, these properties must be scientifically demonstrated. Each plant source must be studied chemically and tested in the clinic. In addition, each source typically undergoes transformations that reflect the specific ways that the source will be clinically applied in the culture in which it will be marketed.

2. ISOLATION AND CONTENT DETERMINATION OF GYMNEMIC ACID

G. sylvestre (*GS*), one of the most powerful medicinal plants [27], is a perennial, woody climber, spread through India and South East Asia, in dry forests up to 600 m height. The plant is large, with opposite leaves, usually elliptic or ovate, and small, yellow flowers. This plant is used in traditional medicine to treat various ailments, especially in India [28]. For example, the leaves are rubbed on infected body parts to cure infections and chewed to suppress the taste for sweet and bitter substances. It is also used in decoctions to cure diabetes and stress-related disorders and is prescribed to treat urinary problems and stomach-ache. Additionally, the leaf extract is applied to treat cornea opacity and other eye diseases. It is used in glycosuria to remove bad odours from breast milk and as an aperitive. Ayurveda states that it is acrid and bitter, digestive and diuretic, anthelmintic and laxative, vomitive, expectorant, stimulant, analgesic, antipyretic and cardiotonic. Furthemore, the leaf extract is traditionally used in treating conjunctivitis, jaundice and leucoderma, hepatosplenomegaly, amenorrhoea and haemorrhoids. The root extract is emetic and used in treating insect bites [27, 28]. In traditional Indian medicine, similarly to other traditional medicine practices, the only part of the plant that is typically used is the leaf. Thus, it is no wonder that most of the articles cited in the literature report the isolation of a saponin-rich fraction from the leaves of *GS,* using a solvent such as ethanol that is pure or has a water content of less than 25-30% (however, in some cases pure water is used on the whole plant). The isolation and characterization of the saponin-rich fraction from indigenous *GS* leaves was conducted with different methods of extraction to optimize the maximum yield. Different parameters such as the concentration of ethanol (70%), the ratio of plant/liquid (1:6), extraction time (3 h), and extraction frequency (twice), were established to obtain the optimum extraction process by infusion [29]. Alternatively, the defatted leaves were extracted under continuous hot extraction in a Soxhlet apparatus with 95% ethanol resulting in the maximum yield of gymnemic acid (6.2%). Moreover, the minimum yield obtained was with aqueous extraction (1.7%). We also used microwave-assisted and releasing-inner ebullition extraction technology, with an extraction time of 8 min, microwave power of 250 W, ethanol concentration of 60%, and solid-solvent ratio of 1:10 [30, 31]. Under the above conditions, the total saponins extraction yield was 98%. When compared to traditional extraction

methods, the microwave-assisted extraction was faster to operate, gave a higher yield and consumed less solvent. By adding water to the alcoholic extract of the leaves, a green soft precipitate was obtained which melted at 68 °C and composed of approximately 0.05% of the leaf material. Hooper named *gymnemic acid* the dark-coloured complex mixture that was obtained from this precipitate with the addition of sulphuric acid [32]. Its soluble extract in ethyl acetate temporarily destroyed the sense of taste for sweet substances. This organic extract was in its turn a mixture of substances, weakly acidic, amounting to approximately 6% of the air-dried leaves then called gymnemic acid (GA) (Table **1**) [33, 34]. In general GA is accompanied by a large number of products of different chemical natures and polarities (for example stigmasterol, α-amyrin, β-amyrin and lupeol acetate). Some protocols have been developed for the partial purification of this fraction as well as for its enrichment in saponins or gymnemic acids and derivatives. The crude gymnemic acid extract (GAE), was separated by affinity chromatography using γ-cyclodextrin as a ligand [35] and enriched with macroporous resins [36]. In particular, the adsorption capacities and adsorption ratios of five types of adsorption resins for the total saponins content were investigated. The concentration and pH of saponins in solution, flow rate and concentration of the elution reagent were optimized using these resins. The results demonstrated that the purity of total saponins increased from 25% to 51% with a recovery of 83%. Next the impurities were eluted with distilled water and 10% ethanol. The adsorbed total saponins were eluted with 50% ethanol. The purity of the resulting total saponins was 56%. Additionally, there is a patent regarding the use of a reverse osmosis membrane with different solvents to obtain a sterile filtrate without a bitter taste. The saponin content can be determined spectrophotometrically [37] or by chemiluminescence [38]. When GA was treated with a mixture of vanillin-glacial acetic acid and perchlorate, it displayed the same colour with a maximum absorption at 545 nm [37]. The linear range was between 40-160 μg, and the method is fast, simple and convenient, and has a high accuracy and stability.

3. PHYTOCHEMICAL ANALYSIS OF EXTRACTS OF *G. SYLVESTRE* R. BR.

Several classes of substances, including steroids, saponins, alkaloids, terpenoids, amino acids, flavonoids, phenolics and essential oils, were the most abundant phytochemicals present in the stem and leaf samples of *Gymnema,* as shown by standard methods that were used for preliminary phytochemical screening of the extracts (Table **2**) [42].

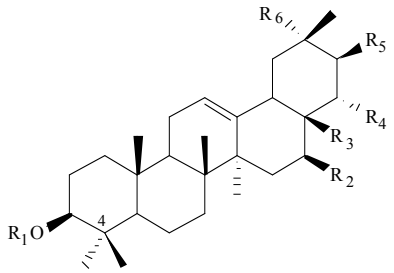
The less polar extract, that was obtained by petrol ether and contained reducing sugars, terpenoids (including a large quantity of limonene), saturated and unsaturated fatty acids (such as hexadecanoic acid ethyl ester), phenolic compounds, hydrocarbons (such as undecane), and saponins [42, 43]. The benzene extract contained reducing sugars, terpenoids and tannins [42]. The chloroform extract contained reducing sugars, tannins, alkaloids (such as 2-isopropylpiperazine), hydrocarbons (such as tridecane), aldehydes (such as butanal), and saturated and unsaturated fatty acids (such as 9-octadecenoic acid) [42-44]. The ethyl acetate extract contained reducing sugars, flavonoids and saponins [42], while the ethanol [45] and methanol extracts [42, 45] contained reducing sugars, terpenoids and tannins; the distilled water extract contained reducing sugars, terpenoids, flavonoids and tannins [42, 45]. Therefore the leaf extract showed moderate concentrations of flavonoids, phenols, saponins, sterols, tannins while the stem extract contained terpenoids, flavonoids, phenols, saponins and sterols [44]. The quality and purity of dry drugs were established by evaluating the ash values. According to Ayurvedic Pharmacopeia [46], the amount of ash in *GS* should not exceed 12%.

4. PHARMACOGNOSTIC ACTIVITIES OF GYMNEMIC ACID AND DIFFERENT EXTRACTS OF *G. SYLVESTRE*

GS has its own chemical composition and includes a large number of chemicals, most of which have their own obvious and known therapeutic activity, while others are considered secondary and some are inert [27-28]. All chemicals, including those that are active and inert, form what is

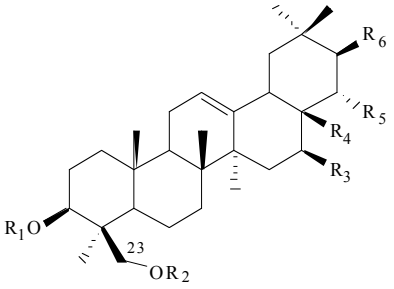
Table 1. Isolation and content determination of Gymnemic acid.

Analysis	Part of the plant	Extract	Ref.
Isolation of saponin fraction (SF)	Leaves	Ethanol	[29, 33, 34]
		Ethanol after defatting	[33]
		Acidic and neutral fractions of ethanol	
		Ethanol without defatting	
		Extraction by microwave	[30, 31]
	Whole plant	Water	[33]
Determination of total saponins	Leaves	-	[37, 38]
Enrichment of SF	-	-	[36, 39, 40]
Partial purification of SF	Leaves	Water	[35, 41]



R_1 = H, β-D-Glucopyranose, β-D-Glucuronic acid, 6-OMethyl-glucuronic acid, 6-O-β-D-Glucopyranosil-β-D-glucopyranose, 6-O-β-D-Glucopyranosil-β-D-glucuronic acid, 6-O-β-Xylopyranosil- 6-O-β-D-Glucopyranosil-β-D-glucopyranose, 3-O-β-D-Glucopyranosil-6-deoxy-α-L-rhamnopyranose;
R_2 = H, OH, O-Acetyl;
R_3 = CH_3, CH_2OH, CH_2O-β-D-glucopyranose, CH_2O-6-deoxy-α-L-mannopyranose, COOH, COO-β-D-Glucopyranose, COO-6-O-β-D-Glucopyranosil-β-D-glucopyranose;
R_4 = H, OH;
R_5 = H, OH, Benzoyl;
R_6 = CH_3, CH_2OH, COOH.

General C-4 Gem-dimethylated oleane structures of triterpenes



R_1 = H, O-Acetyl, β-D-Glucuronic acid, 6-O-β-D-Glucopyranosil-β-D-glucuronic acid, 6-O-β-D-(2-oxo)Glucopyranosil-β-D-glucopyranose;
R_2 = H, O-Acetyl, 3-O-β-D-Glucopyranosil-6-deoxy-α-L-rhamnopyranose;
R_3 = OH, O-Acetyl, Tigloyl, β-D-Glucopyranose;
R_4 = O-Acetyl, OH, H, O-Tigloyl, O-Methylbutanoyl, 6-O-β-D-Glucopyranosil-β-D-glucopyranose, β-D-glucopyranose;
R_5 = H, OH, O-Tigloyl;
R_6 = H, OH, O-Tigloyl, O-Methylbutanoyl, O-Benzoyl.

General 23-hydroxyl-oleane structures of triterpenes

Fig. (1). Structure of the main saponins present in the stem and leaf samples of *Gymnema.*

Table 2. Phytochemical analyses of different extracts.

Study/Analysis	Part of the plant	Extract	Ref.
Phytochemical analyses (qualitative analysis and quantitative estimation of flavonoids, hydrolysable tannins, phenols, saponins, sterols and terpenoids)	Whole plant	Water, methanol, ethyl acetate, chloroform, benzene, petrol ether	[42]
	Aerial parts	Water, methanol and ethanol	[45]
		Methanol	[43, 44, 46]
		Acetone	[44]
		Chloroform	[43, 44]
		Petrol ether	[43]

called a phytocomplex. The action of a phytocomplex may have therapeutic properties that are different from those of one or more of its components taken individually. In comparison to the action of the active principles, phytocomplexes are slower acting, but in conjugation with the active principles, the total action is more complete and less toxic.

4.1. Sweetness-inhibiting Effects

The leaves of *GS* (1 cm^2), when chewed for 1-2 min, temporarily depress sensitivity to sweet and bitter substances [47, 48], and it has been demonstrated that gymnemic acid can completely block the perception of a sweet taste. Numerous studies reported the sweetness-inhibiting effects of *GS* extracts, in particular reducing by an average of 77% the perception of different concentrations of synthetic sweeteners such as acesulfame K, aspartame, sodium cyclamate, and of natural sweeteners such as fructose, glucose, sucrose, stevioside and xylitol [47]. The percentage reduction in sweetness was constant across low, medium and high concentrations of the sweeteners. The effects of *GS* extract (*GS*E), and purified GA were also studied on the taste of glycine and DL-alanine. *GS*E produced the expected depression of tasting sweetness due to the two amino acids and depressions and enhancements for sour, salty and bitter stimuli [49], while the full sweetness-inhibiting effect of GA

appeared after 30 sec and was recovered no earlier than 50 min.

4.2. Hypoglycaemic and Antihyperglycaemic Effects

Hyperglycaemia, an abnormally high blood glucose level, is a hallmark sign of diabetes and its pathophysiology is very complicated and affected by many parameters such as food intake and exercise. Over time, hyperglycaemia can cause microvascular and macrovascular problems, requiring its control in diabetic patients. Although various conventional therapies for diabetes are available on the market, they have many shortcomings, including side effects and high rates of failure. However, herbal extracts are expected to have similar efficacy as with conventional drugs, but without side effects. Various studies present in the literature have reported the use of *GS* extracts in different diabetic models, but the most effective and safest extract for human consumption has not been determined. The water, ethanol, methanol, hexane and chloroform extracts of *GS* were investigated on rats for their hypoglycaemic and antihyperglycaemic potentials [50-52]. In particular, the ethanol extract exhibited the highest hypoglycaemic and antihyperglycaemic potentials (46% and 36%, respectively) [53], while the water extract was less active, reducing the blood glucose levels by 26%, and the methanol extract moderately reduced it only by 12% [50, 53-57]. No significant effects were observed in blood glucose values ingesting the hexane or chloroform extracts. It may be affirmed that the ethanol extract of *GS* leaves has higher hypoglycaemic and antihyperglycaemic potential and should be used as a complementary medicine to treat the diabetic patients by significantly reducing the use of standard drugs. The effect of gymnemic acid (0.4 g/kg of body weight), on the elevation of blood glucose was evaluated in normal subjects after sucrose intake (6 g/kg of body weight). In the *control group*, the fasting blood glucose level (101 mg/dL), was rapidly elevated to 145 mg/dL (44% increase), at 15 min and reached a maximum of 148 mg/dL (47% increase), at 30 min after sucrose administration. Blood glucose levels returned to the basal level after 120 min. In *group 1*, where GA was administered simultaneously with sucrose, the glucose level increased by 23% after 15 min and 33% after 30 min of sucrose administration. The per cent increase in blood glucose level was suppressed to 53 and 68% of the control group at 15 and 30 min after sucrose administration, respectively. Even better results were obtained for *group 2*, where GA was administered at 2 h and 1 h before sucrose and simultaneously with sucrose. The glucose level increased to 13% at 15 min and 20% at 30 min after sucrose administration. The percentages decreased by 31 and 41% when compared to the *control group* and by 58 and 60% compared to *group 1*. These results were confirmed by a study where GA showed dose-dependent hypoglycaemic effects in rats with streptozotocin-induced diabetes mellitus that were loaded orally with 4 g sucrose/kg and 1 to 4 doses of 400 ng gymnemic acid/kg. The results suggest that GA has a suppressive effect on blood glucose levels after sucrose administration.

Thus, GA may have an application in the therapy or prevention of diabetes mellitus and obesity [51, 52]. The high K^+-induced contraction of guinea pig ileal longitudinal muscles and the transmural potential difference in the inverted intestines are the results of inhibition of glucose uptake in the intestines, due to GA and GSE of the leaves, coupled to Na^+ transport processes.

4.3. Gastric Inhibitory Peptide Release Effect

The leaf extracts of GS and purified GA were assayed to evaluate their effects on the increase of Gastric Inhibitory Peptide (GIP), released into the portal vein in response to a duodenal infusion of D-glucose, showing that the increase of GIP was significantly depressed [58]. As GA does not inhibit sweet taste perception in rats, and gurmarin, one of the active components of *Gymnema* leaf extract, does not have any effect on GIP release, this means that not necessarily is the glucose receptor for GIP release identical to the receptor for sweet taste detection. On the other hand, Yoshioka [59] reported that an extract of *GS* leaves and GA inhibited active transport of glucose in rats, showing that the observed inhibition of GIP release by GA could be due to its interaction with the glucose receptor for GIP release, which is similar to the active glucose transport system. These results suggest that a glucose receptor interacts with the leaf extracts of *GS* and purified GA, resulting in the release of immunoreactive GIP and that the glucose receptor for GIP release is not likely to be identical to a glucose transporter.

4.4. Gymnemic Acids and Cholesterol Metabolism

The effect of gymnemic acid on cholesterol metabolism has not been clarified yet. The crude extract from *GS* leaves, the gymnemic acid and a gymnemagenin-rich fraction (GSF), were tested at various concentrations on rats, administering them orally doses of 0.05-1.0 g/kg for 22 days [60]. No significant differences were noted in the faecal excretion of rats dosed with *GS*E, GA or GSF. Body weight and food intakes decreased with the assumption of GSA and GSF, while GSF increased the faecal content of cholesterol, coprostanol (intestinal metabolite of cholesterol), total neutral steroids and bile acids, in a dose-dependent manner. Furthermore, the administration of GA caused the hypertrophy of the stomach wall, suggesting that gymnemic acids irritate

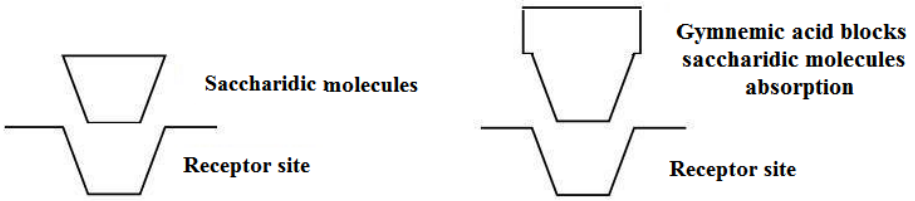


Fig. (2). A general schematic representation of competitive inhibition of receptor site by Gymnemic acid.

the epithelium of the gastrointestinal tract, and in contrast, in animals fed a high cholesterol diet, it decreased serum and hepatic cholesterol.

4.5. Anti-arthritic Activity

The leaf extracts of *GS* were assayed to evaluate their anti-arthritic activity using rats and diclofenac sodium as a standard drug. Among the different extracts analysed, the petroleum ether and aqueous extracts possessed the highest anti-arthritic activity in relation to all parameters considered [61].

4.6. Antimicrobial Activity

GS has been analysed for antimicrobial activity, in particular both crude and pure saponins fractions [45]. The pure saponin fraction was more active than the crude one and was comparable to the activity of chloramphenicol. The gram-negative bacterium *Pseudomonas aeruginosa* was more susceptible to saponins than *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*, and *Klebsiella pneumonia* [62]. Extracts with different solvents of the whole *GS* or of different parts of it were screened for their antimicrobial activity using the agar well diffusion method against *Streptococcus mutans, S. aureus*, *Streptococcus mitis* and *Candida albicans* with doses ranging between 25 and 100 mg/mL. The methanol extract showed the strongest antimicrobial activity. The crude ethanolic extract of *GS* leaves possesses a significant antibacterial activity against *Bacillus pumilis*, *B. subtilis*, *P. aeruginosa* and *S. aureus*. The chloroform extracts of the aerial and root parts were more active than the diethyl ether and acetone ones [63, 64], in particular the root chloroform extract exhibited competitive minimum inhibitory concentration and minimum bactericidal concentration values in the range of 0.04-1.28 mg/mL and 0.08-2.56 mg/mL, respectively [65]. Finally, the hexane extract showed maximum inhibition against *Serratia marcescens* MTCC [66].

4.7. Antifungal Activity

The crude and pure saponin fractions from the leaves of *GS* showed significant antifungal activity, up to 3-fold greater than the activity of the standard drug amphotericin B. *Aspergillus fumigatus* was inhibited more than *Aspergillus flavus* and *Aspergillus niger*, and [67]. It seems to prove that the antimicrobial activity of plant extracts is due to the presence of saponins and that the minimum inhibitory concentration values ranged from 600 to 1,200 mg/L. Saponins may be used in the future as preventive and therapeutic agents against Gram-negative bacteria and fungi.

4.8. Larvicidal Effect

Several plants showed mosquitocidal activity, but few of them have been characterized from a chemical point of view and even less of those that have moved from the laboratory to field use. Insecticides of natural origin may be a viable alternative to those of synthesis, given that more and more insects are resistant to them.

GS seems to be useful to control the population of *Culex tritaeniorhynchus* [44], as demonstrated by the efficacy of its acetone, chloroform and methanol crude extracts. The larvicidal effect of the plant extract was clearly dependent on the concentrations of each extract. No mortality was observed in the control batch larvae maintained in the control medium for 24 h. The experimental larvae were more susceptible to the maximum concentration and the maximum larval mortality of 100.00% was observed with 100 ppm concentration of the acetone and methanol extracts, and 6.9, 10.5 and 15.4% larval mortality was observed for the acetone, chloroform and methanol crude extracts, respectively, at 6.25 ppm. The extracts or isolated pure compounds could be used in stagnant water bodies, to prevent mosquitoes laying and hatching eggs [68].

4.9. Total Phenolic Content and *in vitro* Antioxidant Activity

The methanolic extract of *GS* leaves was analyzed to evaluate the total phenolic content and *in vitro* antioxidant activity using DPPH, hydroxyl radical, nitric oxide radical scavenging and ferric reducing power assays [38, 45, 46]. The total phenolic content of the extract was 2.2 μg/mg of catechol equivalent. The IC_{50} values for the DPPH, hydroxyl radical and nitric oxide radical scavenging assays were 450, 625 and 1,724 μg/mL, respectively. In the ferric reducing power assay, the methanolic extract of *GS* showed an absorbance of 0.13 at 500 μg/mL. The extract showed significant activity due to the presence of phenolic compounds such as flavonoids, tannins and saponins in a concentration-dependent manner.

Rats with diabetes after administration of streptozotocin and a herbo-mineral formulation containing gymnemic acid, showed a significant decrease in blood glucose and urea nitrogen, glycosylated haemoglobin, creatinine, triglycerides, total cholesterol, serum albumin, as well as albumin and creatinine excretion rate [54]. Altered levels of antioxidants like glutathione and superoxide dismutase were restored in the kidney. The experimental results clearly demonstrate that gymnemic acids, which possess potent antioxidant properties, have the ability to prevent the progression of early diabetic nephropathy. These results suggest that the extract may act as a natural antioxidant agent with health benefits.

4.10. Inhibitory Effects on Breast Cancer Resistance Protein

The ethanolic extract of *GS* leaves containing 10.5% gymnemic acid strongly inhibited Breast Cancer Resistance Protein (BCRP)-mediated methotrexate (MTX) at 1 mg/mL. BCRP is an efflux transporter expressed in intestinal epithelial cells, as well as the bile canaliculus, kidney, blood-brain barrier and placenta, and its inhibition increases the systemic availability of various drugs, including methotrexate, topoisomerase I inhibitors, flavopiridol, statins and proton pump inhibitors [69].

4.11. Use as a Weight Loss Aid

A diet with a high content of gymnemic acids can be a valuable aid for the control of body weight. In fact, GA can inhibit oleic acid absorption, as demonstrated by studying the effects of its use in the diet of Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which are obese animal models

with type II diabetes [70, 71]. The studies showed a decrease of feed and water intake (approximately 1/3 and 2/3, respectively), along with body weight. At the end of the experiment, the total cholesterol level decreased by approximately 1/3 and LDL + VLDL cholesterol decreased by approximately 1/2. The blood serum triglyceride level decreased to 1/4 of the OLETF control [72]. Moreover, the results demonstrated that the inhibition by GA was dose-dependent and reversible and that the extent of inhibition and the recovery progress were comparable to that of glucose absorption. Thus, GA promoted body weight loss by its ability to decrease hyperlipidaemia and showed no withdrawal rebound effects on body weight [73].

4.12. Treatment of Dental Caries

The formation and progression of dental caries are closely associated to the presence of different types of Gram-positive bacteria. Primary cariogenic bacteria such as *S. mutans*, *S. aureus*, *S. mitis* and the fungus *C. albicans* form dental plaques that adhere to tooth surfaces through the synthesis of extracellular polysaccharides. Then these bacteria metabolise sugars to organic acids, which are responsible for the demineralisation of tooth enamel. It is therefore clear the need to eliminate the cariogenic bacteria from the oral cavity but keeping in mind that many of these are moderately resistant to antibiotics such as chloramphenicol, clindamycin, and ampicillin. A viable alternative to antibiotics, which are expensive and have side effects such as gastrointestinal problems, would be the use of plants whose extracts are traditionally used for dental hygiene thanks to their powerful antibacterial activity. Among them is *GS*. The effects of various extracts of *GS* in preventing dental caries have been assayed [39] and the methanol extract showed the strongest antimicrobial activity [43]. But also other extracts of *GS* were screened for their particle size and total microbial load, among them the hydro alcoholic extract. This latter one is used in some toothpastes with acceptable findings with respect to efficacy, economic costs, and accessibility [43], confirming the validity of the choice of using natural extracts in treating dental caries.

4.13. *G. sylvestre* in Functional Foods

Currently, *GS* extracts can be used to produce a series of products as functional foods. Normally *GS* ethanol extract or extracts of other plants are used to produce functional foods containing a percentage of *GS* between the 30 and 80%, with a triterpenoid saponin content of 20-70%, and minor quantities of alkaloids, glycosides and anthraquinones [74]. The crude mixture is filtered, the ethanol is recovered, a defoamer and adjuvants are added, the extract is dried and pulverised and then added into capsules or formed into granules or pellets. Using this method, the effectiveness of components of herbal drugs can be extended by emulsification, which is induced by the triterpenoid saponins in water and the low concentration of ethanol [75]. The extract can be used to prepare medicines and health foods with applications in decreasing waist circumference, body weight, obesity index [76, 77], blood pressure, blood sugar [78-80], and blood lipids, or to inhibit the excessive sweetness of moon cake, chocolate, ice cream, etc. for blood sugar control in diabetes [81].

4.14. Effect on Rumen Methanogenesis

About 10% of what ruminants eat is lost or wasted for the production of methane, a greenhouse gas 23 times more potent than carbon dioxide [82]. Therefore, to lower this percentage would mean to improve the efficiency of nutrient utilisation and to make farms more sustainable from an environmental perspective. Once again, the plants can help us, providing compounds to mitigate rumen methanogenesis. Saponins (or tannins) suppress or eliminate protozoa from the rumen and reduce methane and ammonia production.

When *GS* is used as an additive in ruminant feeds it has the potential to suppress methanogenesis, because of the presence of saponins and essential oils, without causing substantial modifications in fermentation parameters [82]. The studies suggest that this material does not affect substrate degradation and is not toxic to ruminal microbes.

5. CONCLUSION

GS is a plant that grows primarily in tropical forests in India and Southeast Asia. The active ingredients of the plant are found in the leaves and roots, which are used in Ayurvedic medicine. For over two thousand years, the leaves of *GS* have been used in the Far East, in particular in India, for the treatment of diabetes. In India this plant is also used to treat snakebites and to relieve intestinal disorders, diseases of the liver and the cold. Indian doctors recommend the use of *GS* as an additive for the treatment of obesity and tooth decay. Its use is more prevalent in India, but the *GS* plant has also been exploited for its numerous properties in Australia, Vietnam and Japan. An active principle that is located in the leaves and roots of *GS* is a mixture of gymnemic acids, which are capable of binding to intestinal receptors that have the task of absorbing sucrose, thus causing a reversible block in sugar absorption and leaving low the blood glucose level. These properties suggest that *GS* is indicated for use in cases of alimentary hyperglycaemia, or a high sugar intake, or in cases of diabetes. The ability of *GS* to inhibit the absorption of sugars and encourage disposal, make *GS* a great aid for those who are overweight and want to lose weight, provided that the excess weight is due to a diet rich in simple sugars and sweets of various types, including potatoes, bread, and pasta.

In addition to limiting blood sugar levels, the *GS* plant also has the effect of nullifying sweet tastes. A small amount of *GS*, placed on the tongue, is able to decrease the sweet taste, leaving unchanged the taste towards bitter, salty and sour substances. This effect can be useful for those who want to fight the desire to consume sweet foods by cancelling the prevailing taste so that they are less attractive. In addition to considerably lowering the absorption of sugar, *GS* is also able to reduce the level of lipids in the blood. It is a plant whose use is suggested for those who suffer from a high concentration of triglycerides and of so-called bad cholesterol in the blood because it considerably reduces the quantity of both. *GS* can even be used to treat cases of water retention as it is recognized as having a mild diuretic effect. Other extensive uses of *GS* include as a treatment for stomach disorders, diseases of the liver and cases of constipation. If that was not enough, *GS* has been used successfully for the treatment of gout and rheumatoid arthritis. Finally, the leaves

Table 3. Biological activities of different extracts.

Entry	Study/Analysis	Part of the plant	Extract	Ref.
A	Effects of the sour, salty, bitter and sweet suppressant	/	/	[47-49]
B	Suppression of glucose absorption. Hypoglycemic and antihyperglycemic activity	-	-	[50]
		-	Ethanol 75%	[53]
		Whole plant	Water	[54, 57]
		Leaves	Water	[51]
			Methanol	[52]
			Ethanol	
			Chloroform	
			Hexane	
C	Effect on glucose-stimulated gastric inhibitory peptide secretion	Leaves	Water gymnemic acid	[58]
D	Anti-arthritic activity	Leaves	-	[61]
E	Antibacterial activity *in vitro* condition	Whole plant	Hexane	[66]
		Aerial parts	Acetone, chloroform, diethylether	[63]
		Leaves	Water, methanol, ethanol, methanol, chloroform, petrol ether	[45]
			Pure saponin fraction	[67]
		Roots	Acetone, chloroform, diethylether	[63]
F	Antifungal activity *in vitro* condition	Leaves	Pure saponin fraction	[67]
G	Larvicidal activity	Leaves	Methanol, acetone chloroform	[68] [44]
H	Antioxidant activity	Whole plant	Water	[54]
		Aerial parts	Water, methanol	[45]
		Leaves	Ethanol, methanol	[46]
		-	-	[38]
I	Inhibitory effects on breast cancer resistance protein	Leaves	Ethanol	[69]
L	Antiobesity effect	Leaves	Saponin-rich fraction	[72, 82]
M	Effect on transdermal delivery of aceclofenac	Whole plant	Methanol	[55, 56]
N	Effects on fecal steroid excretion	-	Saponin-rich fraction	[60]
O	Protective effect on early diabetic nephropathy	Leaves	Methanol	[83]
P	Inhibitory effect on intestinal absorption of oleic acid	Leaves	-	[70, 71]
Q	A method for masking bitter and astringent taste	Natural extract		[84, 85]
R	Identification of dual agonistic novel ligands for insulin receptor	Acetate derivatives of gymnemic acids		[86]
S	Treatment of dental caries	Leaves	Methanol, chloroform, petrol ether	[43]
		-		[39]
T	Effect on rumen fermentation, protozoa population and methanogenesis	Leaves	-	[15]
U	Applications	Natural extract		[74-81]

Extract	Whole plants	Aerial parts	Leaves	Roots
	Entry			
Water	H	H	E	
	B	B	C	
			S	
Methanol	M	H	E	
		B	G/O	
			H	
Ethanol		B	E	
			H/I	
Acetone		E	G	E
Ethyl acetate				
Chloroform		E	G	E
		B	S	
Benzene				
Diethyl ether		E		E
Petrol ether			S	
Hexane	E	B		
Pure saponin fraction			E/F/C/L	

Schema (1). Examined extracts of the different parts of *GS* and the relative biological activities.

of *GS* have also been demonstrated to have significant antibacterial and antiviral properties. *GS* can be taken in capsules or as a herbal tea, and both modes appear to be equally effective. The intake of *GS* in the commonly used doses is no problem for adults and has been deemed very safe. In general, the correct administration of phytotherapeutics poses no risk of toxicity. However, consumers should be aware of the potential toxicity of these products and understand that everything that is natural does not hurt. These products have their own pharmacology, and their effects and the risk of side effects increases when increasing the dose. In particular, for a medicinal plant such as *GS*, it would be interesting and useful to arrive at a precise definition of the fraction called gymnemic acid as well as to accurately delineate the full spectrum of its biological activities, which is a prerequisite for making its use even more extensive. In Table **3** and the schema 1 the biological activities of the examined extracts of the different parts of GS are reported schematically, but large-scale clinical trials have not been conducted to evaluate their safety and efficacy.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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