A Review on *Gymnema sylvestre* and its Major Pharmacological Activities

Submitted By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled “A Review on *Gymnema sylvestre* and its Major Pharmacological Activities” submitted by Humaira Ali (17146059) of Spring 2017 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelors of Pharmacy.

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Ethics Statement

This study does not involve any human and animal trial.

Abstract:

Medicinal plants have always captivated intellectual concerns due to their extraordinary potential for alleviating a wide range of diseases. *Gymnema sylvestre*, with a rich source of active ingredients, is one phenomenal example. This review paper emphasizes the various pharmacological activities that this plant possesses. *G. sylvestre* is credited for excellent anti-diabetic therapy and can also provide several other properties including immunomodulatory, anti-oxidant, anti-obesity, anti-microbial, anti-inflammatory, anti-hyperlipidemic activity. Different parts of *G. sylvestre* consists of many pharmacologically active compounds like gurmarin, gymnemic acids, saponins, flavonol, glycosides, and other important compounds that enables this plant to possess various therapeutic potentials. Traditional medicine systems have employed various portions of the plant, such as the roots, stems, and leaves, as cardiotonic, digestive, diuretic, laxative, stimulant, and uterine tonics. This paper is targeted on the major pharmacological effects that the plant exhibits.

Keywords: *G. sylvestre*; anti-diabetic; phytoconstituents; pharmacological activities.

Dedication

Dedicated to my parents, grandparents and my respectable project supervisor, Dr. Farhana Alam Ripa (Assistant Professor, Department of Pharmacy, Brac University)

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List of Acronyms

Gs	*G. sylvestre*
WHO	World Health Organisation
NIH	National Institute of Health
CHM	Chinese herbal medicine
CMM	Chinese Materia Medica
AgNPs	Silver nanoparticles
GACP	Good cultivation and collection practices
LC-MS	Liquid chromatography- mass spectroscopy
TCL	Thin layer chromatography
R&D	Research and Development
GTT	Glucose Tolerance Test
PMA	Phorbol myristate acetate
NBT	Nitroblue Tetrazolium
LPS	Lipopolysaccharide
DPPH	2,2 - diphenyl -1- picryhydrazyl
AAE	Ascorbic acid equivalents
NADH	Nicotinamide adenine dinucleotide

PMS	Phenazine methosulfate
BHT	Butylated hydroxyl toluene
LDL	Low-density lipoprotein
LDH	lactate dehydrogenase
HFD	High fat diet
TBARS	Thiobarbituric acid reactive substances
MIC	Minimum inhibitory concentration
CHD	Coronary heart disease
TC	Total cholesterol
TG	Triglyceride
HDL-C	High density lipoprotein cholesterol
VLDL	Very low density lipoprotein

Chapter 1

Introduction

1.1 Medicinal plants

Since ancient times, plants have been used as a source of curing diseases, and these plants, with heath maintaining properties, are called by the name 'medicinal plants'. The chronicle of medicinal plants started with the chronicle of human life and the tradition is still practiced widely. For thousands of years, medicinal plants have exhibited usefulness in the improvement of illnesses and also as a root for the discovery of drug lead compounds (Petrovska, 2012). According to an article by Williams & Ahmad (1999), it was during the 19th century when researchers started to pay importance to the process of isolating active ingredients from plants and using them to treat diseases that had no medication or existing medication that were less effective. History says alkaloids such as morphine, strychnine, quinine, etc. were the very first active ingredients to be isolated for usage causing an outbreak in medicinal plant research (Hamburger & Hostettmann, 1991). The necessity to develop new effective drugs was a prime reason for which plants achieved focus and involvement in the scientific and pharmaceutical sector (Taylor et al., 2001). According to the World health organization (WHO) about fourth-fifth of the world's inhabitants use medicinal plants for the prevention of diseases. Statistics before 2006 showed that one-fourth of legal pharmaceuticals either contain plant extracts or are developed modeling plant substances (Debnath et al., 2006). This fraction by 2016 has increased and is estimated that one-third of all medicinal products consist of plant origin (Bahmani et al., 2016). Moreover, the joining of new titles by, for example, Journal of Ethnopharmacology, Phytotherapy Research, Phytomedicine in publications such as Planta Medica, Phytochemistry, and Journal of Natural Products has shown the interest of researchers in medicinal plants and their importance in a health system. In addition, government funding has also been made available in the USA for the NIH (National Institutes of Health) to examine herbs and to conduct clinical trials (Houghton, 2009).

Some plants such as Ginger, Garlic, Green tea, Ginseng, Ispaghol are well known for their nutritional value and are used for therapeutic purposes while other plants serve as raw materials in the drug synthesis and development process. Antibiotics, blood thinners, and laxatives are commonly known drugs to contain active ingredients from plant extracts (Hassan, 2012). Medicinal plants are also confirmed to contain a diverse range of antioxidants and it is shown to be successful in the reduction of lipid peroxidation and therefore, fruitful in the prevention of diseases including atherosclerosis and cardiovascular (Mahmoud & Rafieian-Kopaei, 2012). Cancer is one of the deadly diseases, is demanding new procedures to prevent it from rising. Taxol, camptothecin, vinblastine, stigmasterol, etc., are the anticancer agents found in plants and these constituents are currently in use all over the world (Roy et al., 2019). Nevertheless, medicinal plants have also shown significant improvement in treating metabolic disorders, one serious disorder specifically being diabetes mellitus (Bindu & Narendhirakannan, 2019). Furthermore, medicinal plants have shown to be a great candidate to treat bacterial infections where 78% of new antibacterial agents are natural or molecules derived from a natural product (Lokhande et al., 2007). Herbal medicines have also made their way towards treating psychiatric disorders, for instance, anxiety and depression. In comparison to typical drugs such as antidepressants, herbal plants have shown potential outcomes as psychotropic medicine that too with lesser adverse effects (Saki et al., 2014). By 2003, about 80% of antimicrobial, cardiovascular, immunosuppressive, and anticancer drugs were plant-derived which in terms of sales was estimated to be exceeding US$

65 billion (Pan et al., 2013). On the other hand, Traditional Chinese medicine has been a great influence in the healthcare system that is said to be one of the ancient and persistent. Chinese herbal medicine (CHM) alone, one of the components of Traditional Chinese medicine, covers Chinese Materia Medica (CMM) that embraces thousands of medicinal materials into a pharmacopeia. It is estimated that CHM comprises about 20-50% of the total market of herbal medicine all over the world and holds approximately US$1.025 billion of the international market (Zhou et al., 2019). Medicinal plants are valued because of the pharmacological effects that they possess and there has been increasing verification that they display these actions either by synergistic mechanism or by working in combination to nullify the side effects (Saad et al., 2017). Even so, to ensure safe and effective use of herbal medicine, complete knowledge of their ADME profiles is necessary in addition to their interactions with other synthetic or herbal drugs (Pan et al., 2013). However, to establish their efficacy, studies have been carried out from which some productive results have led to the production of plant-based medicine. It was estimated that the worldwide market value of products made from medicinal plants exceeded $100 billion per annum (Sofowora et al., 2013). Even at present, these plants are used in various countries as a source of medicine as they are safer than synthetic drugs. Another advantage of using medicinal plants as the choice of treatment is that they are inexpensive and easily available (Bahmani et al., 2016). Medicinal plants are the core basis of traditional medicine including Ayurvedic, Unani, Chinese, and other systems that have provided us with quite important medicaments that are still in use for health benefits. Phytomedicines have been proving to be a promising future but to be marked as 'medicine' they will need quality, safety, standardization, and quality control validations. Using them in a non-standardized manner can result in toxicity or other harmful side effects. Thus, millions are spent each year to validate various medicinal plants and to certify that they obey the

standards to be used as a health beneficiary product or as a medicine (Fakim, 2006). Therefore, medicinal plants play a vital role in the pathway of modern medicine and are currently meeting the health needs of millions of people in all cultures (Debnath et al., 2006).

1.2 *Gymnema sylvestre*

G. sylvestre is a medicinal plant belonging to the class dicotyledonous of the Asclepiadaceae family. Asclepiadaceae was considered as a former plant family, according to Angiosperm Phylogeny Group (APG) II classification system, which is recently placed as a sub-family under Apocynaceae (Sanjeet Kumar & Prasad, 2020). Usually, Asclepiadaceae family is known as the Milkweed family because the stems of the plants have a milky, latex-containing liquor. Key characteristics of members of the family include sturdy perennial herbs, shrubs, dainty vines, small trees, and succulent plants. This family consists of about 250 genera and 2,000 species (Britannica, 2013).

Table 1: Taxonomy of family Asclepiadaceae (Sanjeet Kumar & Prasad, 2020)

Kingdom	**Plantae**
Class	Dicotyledonae
Sub-class	Gamopetalae
Series	Bicarpellate
Order	Gentianales
Family	Asclepiadaceae

The plant *G. sylvestre* (Gs) (figure-1) or known as "periploca of wood" in English is native to India and found in the tropical rainforests of the southern and central counterparts (Vijayakumar & Prabhu, 2014). The Genus *Gymnema* includes 40 species and is geographically distributed all over from Western Africa to Australia. A few important species of *Gymnema* includes *G. balsamicum, G. montanum, G. acuminatum (Roxb.), G. latifolium, G. aurantiacum, G. sylvestre R. Br., G. indorum, G. spartum, G. yunnanse* (Kumar et al., 2020). *G. sylvestre* is very well known for its sugar destroying property, which makes it a powerful antidiabetic plant and is commonly used in folk, ayurvedic, and homeopathic healthcare systems (Sharma, 2010). In the global marketplace, this plant is awarded as the second best-selling medicinal herb in the world (Pandey, 2012)

Table 2: Taxonomy of G. sylvestre (Vijayakumar & Prabhu, 2014)

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Gentianales

(Table-2 continued)

Family	**Asclepiadaceae**
Genus	Gymnema
Species	Sylvestre

G. sylvestre is a renowned herb in India because of its effectiveness in treating diabetes mellitus especially and also because it acts as a potent diuretic (Sharma, 2010). The plant is also mentioned in Shushruta, an ancient book on medicine, as a medication for these two health-related issues and in the Indian Materia Medica (Khan et al., 2019; Shanmugasundaram et al., 1983) As type 2 diabetes is increasing alarmingly, it stands as a huge concern for public health (Bais & Choudhary, 2019; Laha & Paul, 2019). People of all ages are suffering from this metabolic disorder (Laha & Paul, 2019). According to Nikalje et al., (2013), by 2010, the number of adults aged between 20-79 years having type 2 diabetes has spiked to 285 million and is expected to climb up to 439 million by 2030. This epidemic disorder, demonstrated by high sugar levels, works as a root for many severe diseases including kidney disease, vision loss, cardiovascular disease, lower-limb amputations, and many more (Bais & Choudhary, 2019). If the condition is not addressed promptly, it might have a detrimental effect on our organs and result in long-term impairment such as diabetic neuropathy (damage of nerve cells), diabetic retinopathy (damage of retinal blood vessels), cataract, diabetic foot infection, bone, and joint problems, etc. Therefore, finding a potential remedy for diabetes mellitus holds great importance in the field of the medical system. In this, *G. sylvestre* plays the role of an exceptional alternative that is cost-effective that can be used in the treatment of type 2 (Laha & Paul, 2019). The utilization of this plant has been rising due to its unique feature of demolishing the taste of sweetness (Rajashekar et al., 2016). This plant is also a treasure of many secondary metabolites and chemical constituents for which it exhibits several pharmacological activities and is used for multiple purposes (Laha & Paul, 2019).

G. sylvestre is commonly known as gurmar (Bindu & Narendhirakannan, 2019). Merging of two Greek words, 'Gymnos'(naked) and 'nema'(thread) gives the generic name 'Gymnema' while the latter part of the name is derived from Latin origin 'sylvestre'(of the forest) (Pramanick, 2016). Including Asclepias geminata, Asclepias geminata, Periploca sylvestris, Gymnema melicida as other scientific names, *G. sylvestre* is called by different names in different languages (Di Fabio et al., 2013).

Figure 1: G. sylvestre (Patel, 2017)

Table 3: Different names of G. sylvestre in different languages (Di Fabio et al., 2013).

Languages	Names
English	Gymnema, Cowplant, Australian cowplant, Gurmari, Gurmarbooti, Gurmar, Periploca of the woods, Meshasringa, Gemnema Melicida, Gimnema, Gur-Mar, Gymnema montanum, Gymnéma, Gymnéma Sylvestre, Miracle plant, Periploca sylvestris, Shardunika, Vishani, Ram's horn, Miracle fruit, Merasingi, Small Indian ipecac, Sugar destroyer
Bengali	Mera-Singi
Sanskrit	Meshashringi, Madhunashini, Ajaballi, Ajagandini, Bahalchakshu, Karnika, Chakshurabahala, Kshinavartta
Marathi	Kavali, Kalikardori, Vakundi
Hindi	Gurmar, Merasingi
Gujrathi	Dhuleti, Mardashingi
Telugu	Podapatri
Tamil	Adigam, Cherukurinja, Sarkarikolli

(Table-3 continued)

Kannada	**Sannager-asehambu**
Malayalam	Chakkarakolli, Madhunashini

G. sylvestre is documented to possess several pharmacological activities including antimicrobial, anti-hyphal, anti-hypercholesterolemic, hepatoprotective, hypolipidaemic, antihelmintic, and free radical scavenging activities (Di Fabio et al., 2013; Sharma, 2010). The plant is also used in the treatment of asthma, eye complaints, inflammations, family planning, metabolic syndrome, cough, and malaria. (Kanetkar et al., 2007; Patel, 2017). In addition, *G. sylvestre* is used as an antidote to snake bites, digestive stimulant, laxative, appetite suppressant, diuretic, and an ulcer (Patel et al., 2012; Patel, 2017). Dyspepsia, jaundice, haemorrhoids, cardiopathy, bronchitis, and leucoderma are some of the other health conditions in which this medicinal plant is believed to be useful (Sharma, 2010). Moreover, it is known to act as a botanical pesticide to caterpillar, Prodenia eridania and to have the potential to prevent dental caries caused by Streptococcus mutans and in skin cosmetics (Kanetkar et al., 2007). People in a few districts of India are known to have the habit of chewing leaves of *G. sylvestre* in the morning to obtain clear urine and to reduce glycosuria. Vaids, practitioners of Ayurvedic medicine, in Bombay and Madras suggest the leaves of this plant as a medication for furunculosis (a deep infection of the hair follicle) and hyperglycemia (Vijayakumar & Prabhu, 2014). According to Mey (2019), due to its potent antidiabetic and antiobesity property, this plant is used to make supplements and tea preparations. Latest health products made from *G. sylvestre* are also reported to be used in Europe, Asia, and North America.

Chapter 2

Plant description

In this section, the geographical distribution, morphology and anatomy, and the cultivation and micropropagation of *G. sylvestre* will be described.

2.1 Geographical distribution

Each plant has a specific geographical distribution and is native to a country or nation. This geographical distribution may get modified due to climate change and cause a negative impact on the biodiversity of the given plant (Kosanic et al., 2018). The plant responds to this alteration or changed by adapting, moving into a safe direction where the climate is more appropriate, may have elevations to higher latitude, or worst-case scenario, they may get defunct regionally, nationally, or globally (Palmer et al., 2015). When it comes to medicinal plants, geography plays an important role as geographic location can put an impact on the chemical composition of a plant (Thanh et al., 2018).

G. sylvestre is a woody climber plant (figure-1) that is native to India, Malaysia, Srilanka, Indonesia, Japan, Vietnam, and the southwestern region of the People's Republic of China (Sharma, 2010). The plant itself is slow-growing and is well distributed in Asia, Australia, and tropical Africa (Kanetkar et al., 2007). Banda, konkan, Western Ghats, Deccan are also found to be a habitat for this plant (Sharma, 2010). It prefers to grow at an elevation of 300-700m and is capable of spreading in coastal plains, scrub jungles, and copse. (Vijayakumar & Prabhu, 2014). The climate of high humidity favors the plant to its best and thus *G. sylvestre* is mostly seen in

Figure 2: Geographical representation of G. sylvestre around the world (Parveen et al., 2015)

tropical or subtropical regions and forest hills. As it is a climber, it grows with the help of external support (Tiwari et al., 2014).

2.2 Morphology and anatomy

Pramanick (2016) described the plant as a large perennial that can be found running overhead of the trees that grow in the dry forests of regions of Asia. The plant reaches up to 600 m in height in the forests of India. *G. sylvestre* emerges to be highly branched and is more or less pubescent with young stems and branches (Khan et al., 2019).

Figure 3: Leaves of G. sylvestre (Mey, 2019)

a) Characteristics of the leaves of *G. sylvestre* are described below:

- The leaves are simple have short petiole and are green in color (Gurav et al., 2007; Sharma, 2010).
- They are opposite and elliptic or ovate (Sharma, 2010).
- The size of the leaves is about 3.2- 6.5 cm in length and 3-16 mm in diameter (Patel, 2017).
- The leaves have a distinctive smell and a light bitter and astringent taste (Sharma, 2010).

- The transverse section of the leaves appears to have an upper and lower cuticle-covered epidermis (Patel, 2017).
- The vascular bundle of the plant is fan-shaped which is located at the center (Patel, 2017).
- The leaf of these plants also contains trichomes (Patel, 2017).
- Triterpene saponins are the organic chemicals found in the leaves of *G. sylvestre* (Kanetkar et al., 2007).
- The presence of proteins, sugars, alkaloids, and fats are also detected in the extracts of the leaves (Nikalje et al., 2013).
- Nonetheless, the percentage of methanolic extractive value in the leaves was traced to be around 6.25 which was highest in comparison with the percentage found with stem and roots (Nikalje et al., 2013).

Figure 4: Flower of G. sylvestre (Ken Fern, 2014)

b) Characteristics of the flowers of *G. sylvestre* are described below:

- Flowers are small and yellow (Gurav et al., 2007).
- The follicles are cylindrical and lance head-shaped which are up to 3 inches in length (Gurav et al., 2007).

- The lobes of the calyx are long and ovate (Sinha & Mondal, 2017).
- Petals are bell-shaped and pale yellow with each petal consisting of 5 fleshy scales (Sinha & Mondal, 2017).
- The membranous tip of the flower consists of 2 carpels and 5 pollinia (Sinha & Mondal, 2017).
- The plant produces flowers from June to August that sums up to a 3-month flowering time (Sinha & Mondal, 2017).

Figure 5: Roots of G. sylvestre (Devi & Srinivasan, 2008)

c) Characteristics of roots of *G. sylvestre* are described below:

- This plant comprises hairy roots (Rajashekar et al., 2016).
- The transverse section of roots has a circular borderline and a curvy appearance (Nikalje et al., 2013).
- Microscopic study shows that it has an epidermis which is enveloped with a layer of the cuticle that has a good thickness and it is a multilayered structure with parenchymatous cells (Nikalje et al., 2013).

- Endodermis and pericycle, xylem accompanied by phloem, and pith are present in the roots of this plant (Nikalje et al., 2013).
- The roots of this plant serve as a good source of active ingredients (Rajashekar et al., 2016).
- They are also found to contain proteins, sugars, alkaloids, and fats (Nikalje et al., 2013).

Figure 6: Stem of G. sylvestre (Ali, 2020)

According to Liu et al., (2014) the properties of the chemical constituents found in the leaves and the stems of the plant are not identical. The stem contains a unique triterpenoid saponin which is an absence in the leaves of the plant. Moreover, the stem part of *G. sylvestre* is used in the production of silver nanoparticles (AgNPs) which plays a great role in the enhancement of the healthcare system. The invention of organic AgNPs from the stem has made the synthesis process economical and eco-friendly (Renganathan et al., 2017).

d) Characteristics of the stem of *G. sylvestre* are described below:

- Same as roots, the marginal line of the stem is also round when cut in the transverse section (Sharma, 2010).
- Microscopic characteristic shows it has a thick epidermis and multicellular trichomes (Sharma, 2010).
- The cork consists of cortical cells and the phloem well mimics a perforated wall containing phloem parenchyma (Sharma, 2010).
- The xylem is a continuous cylinder with thin medullary rays running through it (Sharma, 2010).
- Extracts of stem showed the positive result to contain proteins, sugars, alkaloids, and fats but starch was absent (Nikalje et al., 2013).

This plant produces one or two fruits that are dark green in color and seeds are oval. Its fruiting time starts in October (Karale & Karale, 2017).

2.3 Cultivation and micropropagation

As *G. sylvestre* is said to be very beneficial for managing obesity and helps in controlling diabetes as well, the increased demand for this plant has been an issue that had rendered the plant to be an endangered species. The most vulnerable species is *Gymnema sylvestre* R. Br. Species. The species has very low seed variability and over-exploitation by farmers has caused the plant to retreat from their very own natural habitat (Rajashekar et al., 2016). However, as a result of ineffective agro techniques, the numerous varieties and populations emerging in various phytogeographical regions deserve the attention of scientific investigators (Singh et al., 2008). In addition, this plant is one of the most popular medicinal herbs in the international market needed a cost-effective and easy cultivation method to keep up with demand. While scientists were seeking alternatives for saving the plant, different methods were used for cultivation. According to World Health Organization (WHO) guidelines, factors such as cultivation technique, harvesting method, collection method, post-harvesting process, transport, and storage techniques ensure good raw material and finished products. A general guideline of good cultivation and collection practices is (GACP) given for the collection and regulation of medicinal plants and herbs. (WHO, 2003).

G. sylvestre can flourish in a variety of soil conditions but a well-drained red loamy soil or moderate black soil extremely favors the plant. During cultivation, it is suggested to grow the plant in dry fields and to avoid waterlogging which the plant is unable to withstand. For optimal growth, a tropical climate with heavy to average rainfall is mandatory (Singh et al., 2008). Germplasm conservation, seed pretreatment, seed germination, vegetative propagation, and hairy root culture were some of the options (Pandey, 2012; Rajashekar et al., 2016; Singh et al., 2008). Pandey (2012) summarized the important processes that were crucial for the productive cultivation of this plant. During seed germination, seeds must be given cold water treatment before sowing them in the mixture of sand, soil, and Farm Yard Manure. May is found to be the best month for sowing whereas for vegetative propagation the beneficiary months are March and July. The plant roots exhibited the highest rooting results when propagated with hormones than without. Crop maintenance should include weeding, hoeing, and irrigation daily and after 24 months of planting, harvesting should be undertaken twice a year by hand plucking the leaves.

Moreover, it is established that *G. sylvestre* in different region consists of slightly different chemical constituents. Extract of the plant from different geographic locations showed inconsistent results when examined under liquid chromatography-mass spectroscopy (LC-MS). Chemical variation may have an impact on its pharmacological function, efficacy, and standardization which is a major concern as it is used as a medicinal plant (Thanh et al., 2018). Therefore, the germplasm of species from all territories must be screened so that the best one can be used for bulk production (Singh et al., 2008).

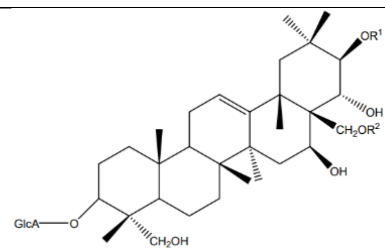
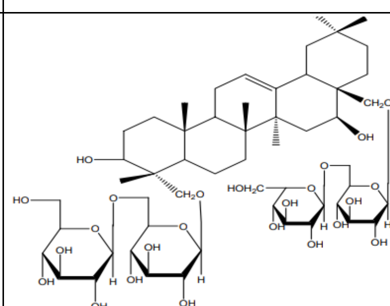
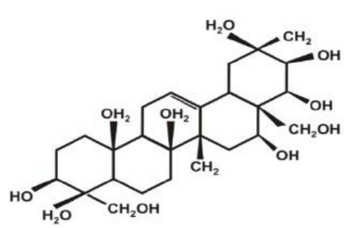
The active principles of the plant can also be preserved using the hairy root culture option. *G. sylvestre* is tough to cultivate due to problems like low rooting ability and unhurried germination rate thus hairy root culture can be the solution to overcome all the problems related to cultivation. The technique is said to be more beneficial compared to other cell suspension culture types because it ensures genetic stability, rapid doubling time, maintenance ease, and lastly, autotrophic (Rajashekar et al., 2016).

Chapter 3

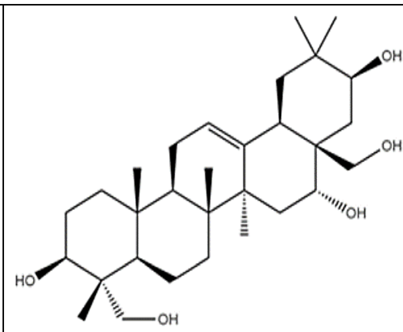
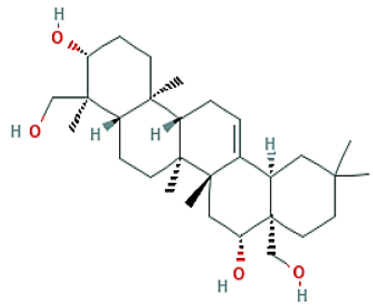
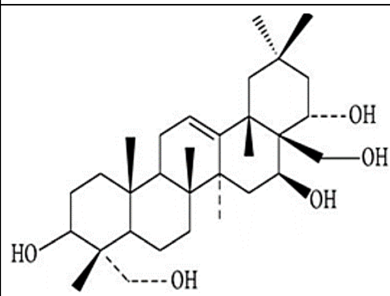
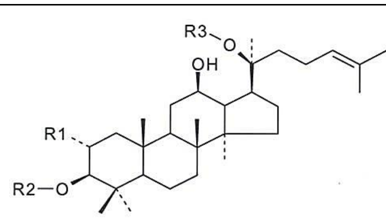
Active Constituents

A number of researchers have found and reported many phytochemical constituents present in the plant. The plant is reported to contain a variable number of classes of secondary metabolites. These included flavonoids, phytosterols, anthraquinones, and a mainly complex mixture of saponins called triterpene saponins (Karale & Karale, 2017; Mey, 2019). Among them, triterpene saponins are found to be the main components exhibiting the antisaccharine effect (Gurav et al., 2007). *G. sylvestre* contains triterpene saponin which are oleanane and dammarene type saponins (Mayra, 2013; Sahu et al., 1996). Oleanane saponins isolated from gymnema are gymnemic acids, gymnema saponins, gymnemagenin, gymnestrogenin (pentahydroxytriterpene) 23-hydroxylongispinogenin and gymnemanol while dammarene saponins are gypenoside and gymnemasides. Gymnemic acids are triterpene glycosides with an aglycone gymnemagenin as the core structure and this compound is distributed in all parts of the plant (Gurav et al., 2007; Tiwari et al., 2014) This aglycone gymnemagenin, which is a D-glucoronide of hexa-hydroxytriterpene, when attached with different sugars and several ester groups give rise to different gymnemic acids. These acids are made of various chemical compounds of the same general formula and they are marked as the chief active component with an anti-diabetic effect. Four other tritepenoid saponins isolated from the leaves are named gymnemasin A, B, C and D. Prosapogenin is also found to be a saponin in gymnema (Gurav et al., 2007; Laha & Paul, 2019; Sharma, 2010). Sinsheimer et al. (1970) conducted a study in which they isolated gymnemic acids from the plant by direct thin layer chromatography (TLC) in different solvent systems. They started the experiment by obtaining a crude gymnemic acid mixture. Defatted leaves were used and extraction was done by 95% ethanol. Acetone extraction: silicic-acid chromatography was then carried out for the separation of the acids. They determined the presence of five major gymnemic acids which they named gymnemic acids A-D and V. Shoot tips of the plant have the highest content of gymnemic acid, whereas seeds contain the least (Tiwari et al., 2014).

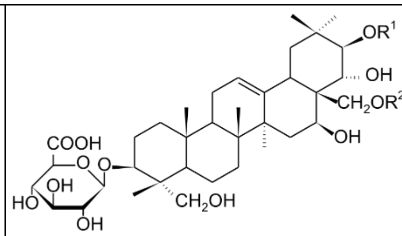
Table 4: Classification of triterpenoid saponins of Gymnema

CLASS	CLASSIFICATION	BASIC STRUCTURE	REFERFENCES
Oleanane Saponins	Gymnemic acids I-XVIII, Gymnemic acids A,B,C,D and F		(Tiwari et al., 2014) (Tran et al., 2020) (Singh et al., 2008)
	Gymnema saponins I-V		(Mey, 2019)
	Gymnemagenin I-V		(Vaidya, 2011)

(Table-4 continued)

	Gymnestrogenin		(Dash et al., 2018)
	23-hydroxylongispinogenin		("PubChem Compound Summary for CID 13322806, 23-Hydroxylongispinogenin.," 2021)
	Gymnemanol		(Dash et al., 2018)
Dammarene saponins	gypenoside XXVIII, XXX, VII, LV, LXII, LXIII		(Laha & Paul, 2019) (Aktan & Davies, 2005)

(Table-4 continued)

	Gymnemosides A, B, C, D, and F		(Tiwari et al., 2014) (Vaidya, 2011)

Gurmarin, which is an important 35 amino-acid peptide is discovered from the plant and is reported to have a molecular weight of 4209 exhibiting sweet suppression property to its best when in a position near to its isoelectric point (Karale & Karale, 2017; Tiwari et al., 2014; Tran et al., 2020) Aerial parts of *G. sylvestre* are a good source of flavonol glycosides that includes kaempferol-3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-β galactopyranoside, kaempferol-3-O-robinobioside, rutin, quercetin-3-O-robinobioside, and tamarixetin-3-O-robinobioside (Gurav et al., 2007; Mey, 2019; Sharma, 2010). Test for the presence of flavonoids was done by Kumar et al., (2020). They took 0.5 ml of plant extract and added 5 ml of distilled water in a test tube. It was then mixed and filtered. Then they added 5 ml of dilute ammonium solution and then conc. sulfuric acid which resulted in a yellow color formation. This color formation confirmed the presence of flavonoids in the sample.

Nikalje et al., (2013) found the presence of alkaloids in the leaf, stem, and roots of the plant. They took sections of the plant and performed tests with four different reagents to detect alkaloids. Reddish-brown precipitate with Wagner's Reagent, precipitate formation with Dragendroff's

Reagent, formation of cream color with Mayer's Reagent, and yellow precipitate with Hager's Reagent unquestionably specified alkaloids to be present. Out of the alkaloids, gymnamine and its hydroxyl derivative and 8-hydroxy gymnamin were reported (Mey, 2019). Hentriacontane, pentatriacontane, phytin, δ-quercitol, lupeol, β-amyrin-related glycosides, acidic glycosides, anthraquinone derivatives, and stigmasterol are also among the unique constituents of *G. sylvestre* (Di Fabio et al., 2013; Gurav et al., 2007; Sharma, 2010; Vijayakumar & Prabhu, 2014). Kumar et al., (2020) determined the presence of sterols and cardiac glycosides. For detecting steroids, 0.5 ml of sample extract was used and 3 ml chloroform was added to it. After filtration, 2 ml of conc. sulfuric acid was added that formed a reddish-brown ring at the interface. The presence of cardiac glycosides was also confirmed with the same color ring formation. They used 0.5 ml of an extract with 0.2 ml glacial acetic acid and added 3.5% ferric chloride in drops. This mixture was then layered with 1 ml of conc. sulfuric acid.

Liu et al., (2014) studied the phytochemicals found in the stems of *G. sylvestre* and isolated seven compounds. Other than conduritol A, stigmasterol and lupeol, stigmasterol-3-O-β-D-glucoside, the sodium salt of 22α-hydroxy-longispinogenin-3-O-β-D-glucopyranosyl-(1→3)-β-D-glucurono-pyranosyl-28-O-α-L-rhamnopyranoside, oleanolic acid-3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, and the sodium salt of 22α-hydroxy-longispinogenin 3-O-β-D-glucuronopyranosyl-28-O-α-L-rhamnopyranoside were isolated.

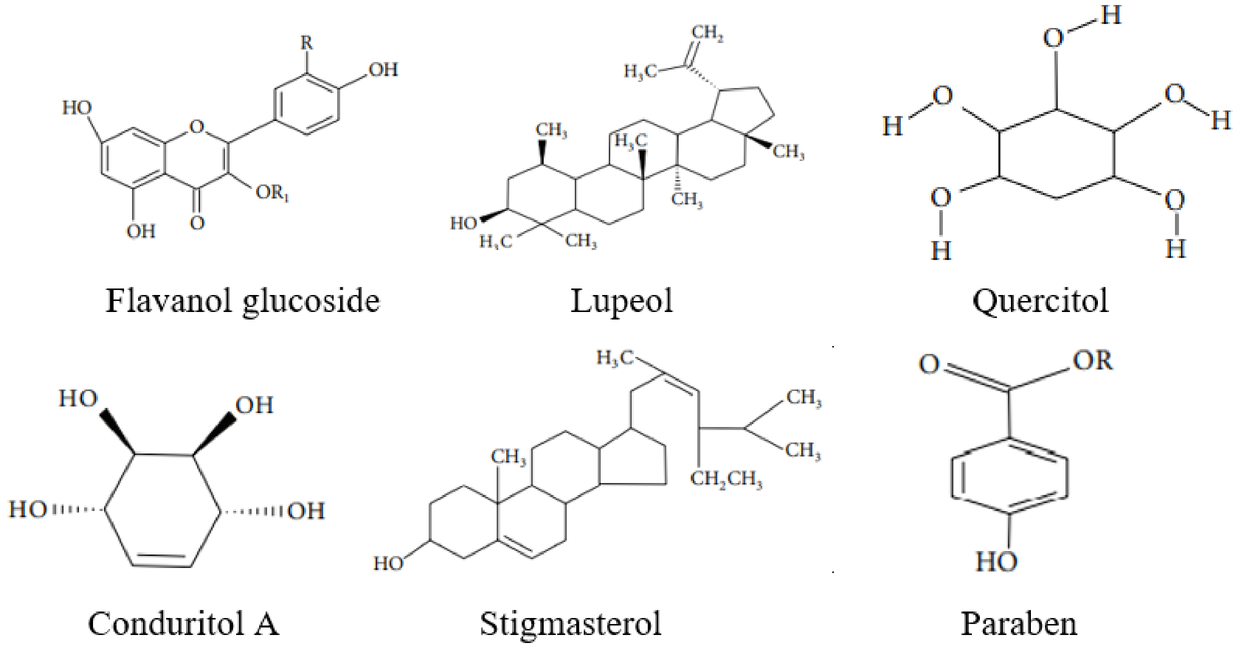


Figure 7: Structures of few phytoconstituents found in gymnema (Tiwari et al., 2014).

Gurav et al., (2007) and Sharma (2010) reported many other valuable constituents of the leaves that included resins, albumin, chlorophyll, tartaric acid, formic acid, butyric acid, inositol alkaloids. They also reported organic acid 5.5%, parabin, calcium oxalate, 7.3%; lignin, 4.8%; cellulose, 22% to be present. Other than these, ascorbic-acid, beta carotene, betaine, choline, and niacin in also found as phytochemical compounds (Vijayakumar & Prabhu, 2014). According to Sharma et al., (2017), this plant accommodates crude fat, crude protein, crude fiber, and carbohydrates. Test for the determination of proteins and carbohydrates was conducted by Kumar et al., (2020). For confirming the presence of proteins, they used 1 ml of the extract, 500µL of copper sulphate solution, and 500µL of 5% sodium hydroxide solution. The resulting mixture gave a purple violet color. On the other hand, for carbohydrates, they suggested adding 1 or 2 ml of the extract with 1-2 drop of ɑ-naphthalol and then adding 2 ml of conc. sulfuric acid. The formation of a violet ring assured the presence of carbohydrates.

Table 5: Important phytoconstituents of G. sylvestre

Phytoconstituents	Percentage
Organic acid	5.5% (Gurav et al., 2007; Sharma, 2010)
Calcium oxalate	7.3% (Gurav et al., 2007; Sharma, 2010)
Lignin	4.8% (Gurav et al., 2007; Sharma, 2010)
Cellulose	22% (Gurav et al., 2007; Sharma, 2010)
Crude fat	5.80% (Sharma et al., 2017)
Crude protein	10.94% (Sharma et al., 2017)
Crude fiber	11.50% (Sharma et al., 2017)
Total carbohydrates	54.89% (Sharma et al., 2017)

G. sylvestre is found to be a good resource for tannins and phenols. The existence of tannins was ensured by taking 0.5 ml of the sample extract and diluting it with 5 ml of distilled water. The addition of 1% ferric chloride resulted in the emergence of deep green color. The presence of tannins was also assured by Salkowski test (Doss, 2009; Kumar et al., 2020). Phenols as a chemical constituent were revealed by using 1 ml of the extract and adding 2 ml of distilled water to it. At the next step, 2 drops of 10% $FeCl3$ were introduced, and as an outcome blue or green color indicated the presence of phenols (Kumar et al., 2020).

The plant is also very rich in mineral contents. It contains calcium, magnesium, chromium, zinc, copper, and iron. The calcium content of this plant is found to be the highest while least for chromium (Sharma et al., 2017).

Table 6: Minerals of G. sylvestre (Sharma et al., 2017)

Minerals	**Mg per 100g**
Calcium	1542.63
Magnesium	592.40
Chromium	2.70
Zinc	21.80
Copper	12.71
Iron	36.91

The amount of minerals listed above is an average of three determinations and is determined from *G. sylvestre* powder.

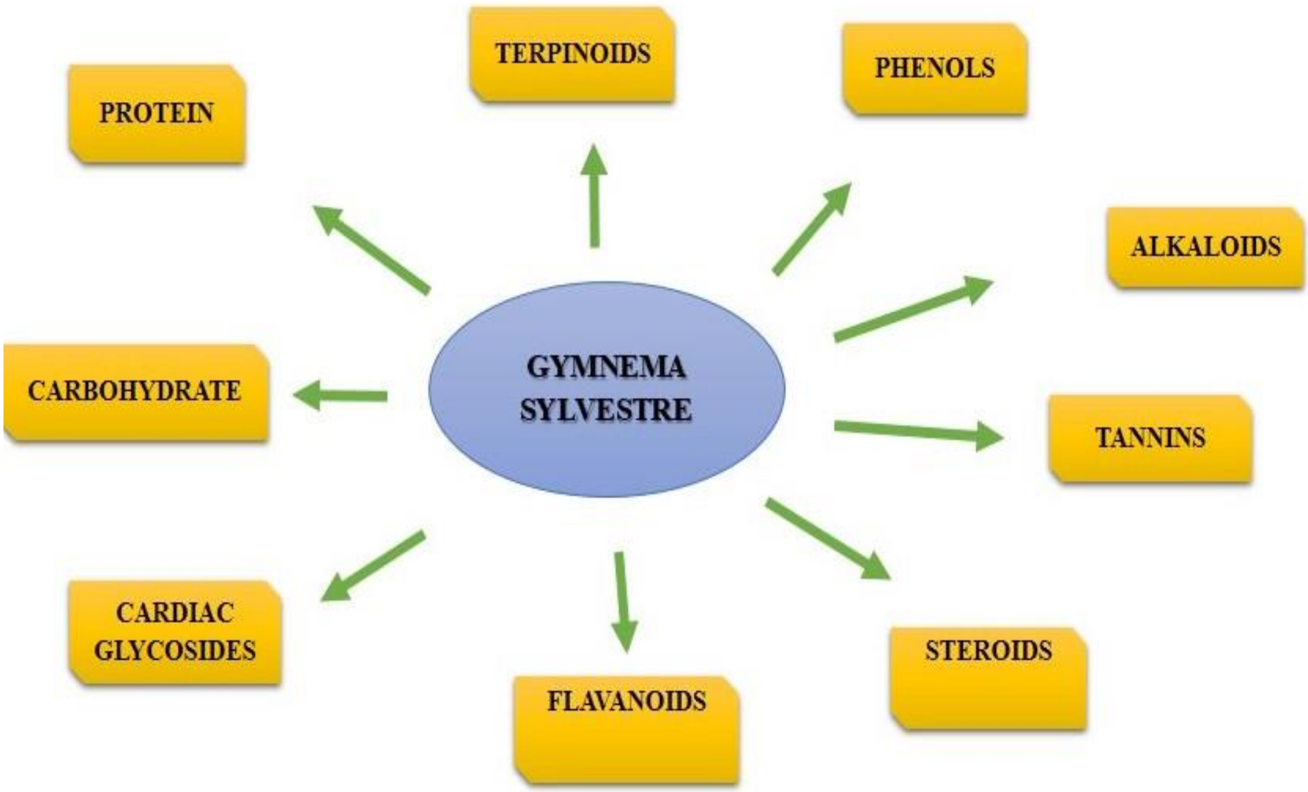


Figure 8: Classes of Phytochemical constituents present in G. sylvestre (Khan et al., 2019).

Table 7: Biological activities of some important active constituents.

Compounds	**Biological activities**
Gymnemic acids	Anti-diabetic activity (Laha & Paul, 2019)
Tannins	Anticarcinogenic, antimutagenic, antioxidative properties (Chung et al., 2016)
Beta-Carotene	Anti-oxidant activity, anti-cancer activity (Milani et al., 2017)
Ascorbic-acid	Anti-oxidant activity (Mandl et al., 2009)
Quercitol	Anti-diabetic activity (Bhushan et al., 2010)
Conduritol-A	Hypoglycemic activity (Laha & Paul, 2019)
Niacin	Lipid disorders and cardiovascular disease (Kamanna & Kashyap, 2008)
Choline	Antimanic, Cardiodepressant, Cerebrotonic, Hepatoprotective (Vaidya, 2011)
Betaine	Antigastritic, Antihomocystinuric, Ethanolytic, Hepatoprotective (Meririnne et al., 2018; Vaidya, 2011)
Stigmasterol	antiosteoarthritic, antihypercholesterolemic, cytotoxicity, antitumor, hypoglycaemic, antimutagenic, antioxidant, anti-inflammatory, and CNS effects (Chaudhary et al., 2011)

Chapter 4

Major pharmacological activities

4.1 Anti-diabetic activity

The glucose level in blood should be maintained within an equilibrium as it plays an important role in survival. There are various and diverse factors that influence the level of glucose. The blood glucose content is kept under strict limitations by the mechanism of homeostasis of our body (Szablewski, 2011). This mechanism includes two hormones, insulin, and glucagon. Insulin and glucagon both are peptide hormones and they are secreted from the cells of the pancreatic islets of Langerhans, insulin from beta cells whereas glucagon from alpha cells (Rix I et al., 2019; Wilcox,

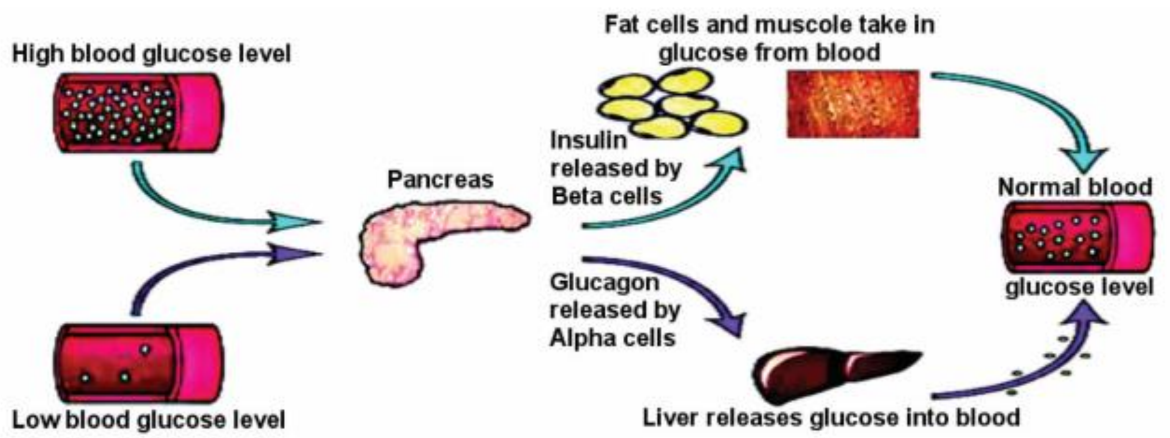


Figure 9: Homeostasis of blood glucose (Manca et al., 2011)

2014). When our body falls short of glucose, the alpha cells of the pancreas releases glucagon. This hormone then stimulates the liver cells causing glucose to be released into the blood (Manca et al., 2011). Inversely, after a meal, glucose is absorbed by the stomach and intestine, then reaches the bloodstream. As blood glucose level increases, the secretion of insulin is stimulated (Szablewski, 2011). This hormone then promotes the conversion of glucose to fat, helps in storing glucose as glucagon, modulates carbohydrates, and takes part in protein metabolism thus lowering the level of glucose to equilibrium (Wilcox, 2014).

When this hormone insulin is not released properly or does not function correctly after secretion, it results in a hyperglycemic condition. This condition abiding for a longer period leads to diabetes mellitus which is represented by a group of metabolic diseases (State & Biology, 2013). Diabetes mellitus is titled the epidemic of the century and is the most prevalent endocrine condition, affecting over 100 million people worldwide (Grover et al., 2002; Kharroubi & Darwish, 2015). Type I diabetes and type II diabetes are the two most common types of diabetes. Type I diabetes is insulin-dependent diabetes that occurs owing to autoimmune destruction of pancreatic beta cells by T-lymphocytes of the immune system. In this condition, insulin production by the pancreas is no longer possible (Manca et al., 2011). On the other hand, type II diabetes is non-insulin-dependent. In type II diabetes, cells fail to respond normally to the hormone insulin which is known as insulin resistance. Sometimes this condition is also worsened by lack of insulin due to beta-cell malfunction (State & Biology, 2013).

Treatment with *G. sylvestre* is shown to be effective in both types I and type II diabetes (Singh et al., 2008). Shanmugasundaram et al., (1990b) carried an investigation where 27 patients with type I diabetes were given 400mg/day while taking insulin therapy. During this study, it was found that the fasting blood glucose, glycosylated haemoglobin and glycosylated plasma protein of the patients decreased gradually and so the patients required lesser insulin dose. As in type I diabetes the insulin-producing cells are destroyed, the possible mechanism that worked is increasing the count of beta cells or islets causing regeneration of the islets of Langerhans. Shanmugasundaram, et al., (1990a) conducted another study on albino rats to check the efficacy of *G. sylvestre* on blood sugar levels. The rats were treated with streptozotocin (55mg/kg) to generate diabetes. 35 days after the injection was administered, half of the normal rats and diabetic rats were treated with *G. sylvestre* extract 20mg/day. During the end of the experiment, fasting blood glucose was tested every 5 days and on the 20^{th} day the test results for rats treated with alcoholic extract (3% yield w/w relative to starting material) were within the normal range. It was noted that using *G. sylvestre* leaf extract elevated the secretion of insulin and also the number of beta cells. Furthermore, the results of the experiment regulated by Shanmugasundaram et al., (1983) on alloxan diabetic rabbits revealed that *G. sylvestre* also increases the activity of insulin-sensitive enzymes. Hexokinase and glyceraldehydes 3-phospahte dehydrogenase are two enzymes needed in the catalyzation of glucose (Granot et al., 2013; Tunio et al., 2010), In the study, Shanmugasundaram et al., (1983) observed an enhanced function of these enzymes such as glucose 6-phosphate dehydrogenase in diabetic rabbits treated with *G. sylvestre* compared with untreated diabetic rabbits.

In the investigation of Paliwal et al., (2009), the outcome of administering *G. sylvestre* leaf powder to type II diabetic patients were seen. The patient's fasting and postprandial blood glucose levels were examined for a month and a notable decrease in both glucose measurements with a p-value of less than 0.01, which is statistically significant, was observed. Patients taking oral anti-hyperglycemic conventional medicine were tested for 18-20 months by giving capsules containing ethanol extract of *G. sylvestre* leaves. Taking 400mg per day resulted in reduced sugar levels, glycosylated hemoglobin, and glycosylated plasma proteins. 21 patients had to take less dosage of conventional medicine and five among 22 diabetic individuals were able to stop taking their regular medication and maintain blood glucose levels with only *G. sylvestre* supplements (Baskaran et al., 1990). In addition, dose-dependent effects of oral administration of aqueous leaf extract (50, 100, 200, and 400 mg/kg) to normal and streptozotocin-diabetic rats were observed and showed lowering of blood glucose levels. Furthermore, the glucose-fed rats' hepatic glycogen content was dramatically reduced by a water-soluble component of the plant's alcoholic extract (Grover et al., 2002). When insulin-resistant rats were treated with aqueous extract of the plant, improvement in altered glucose, insulin, and lipid profile was documented. Pretreatment with *G. sylvestre* helped to avoid the development of insulin resistance and the complications that come with diabetes mellitus (Huilgol & Yendigeri, 2015).

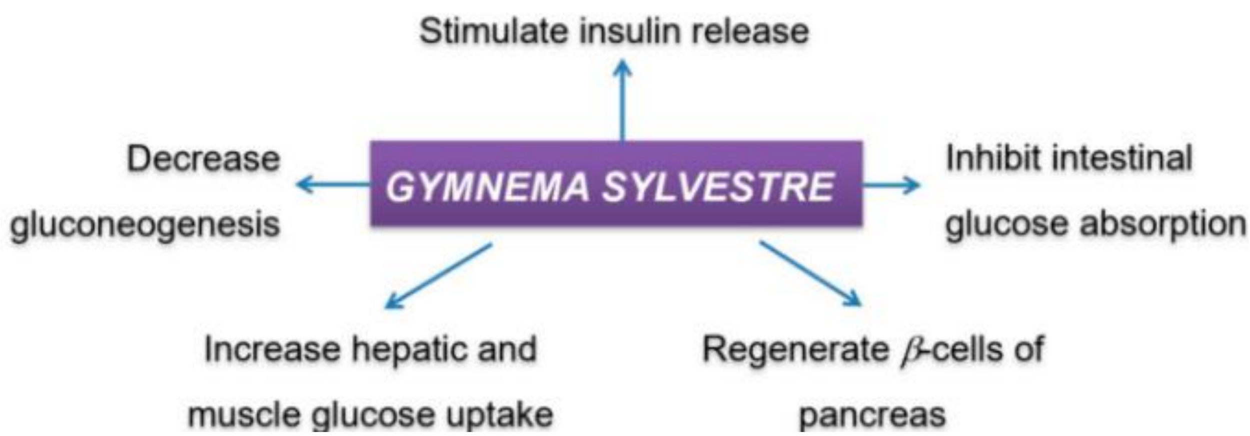


Figure 10: Mechanism by which G. sylvestre exerts anti-diabetic activity (Tran et al., 2020)

The anti-diabetic action of *G. sylvestre* has been attributed to a number of mechanisms, where the main role is thought to be played by gymnemic acids (Tran et al., 2020). Mechanism by which these acids exert hypoglycemic activity is that they can obstruct glucose absorption in the blood. Gymnemic acid’s atomic arrangements are comparable to sugar molecules, which fill the taste receptors in the taste buds and prevent them from being activated by the sugar molecule in the meal thus cutting the sugar craving (Bhatt & Khobra, 2019; Thakur et al., 2018; Tiwari et al., 2014). It also inhibits sugar molecules from being absorbed by the intestine by binding and blocking the receptor present in the external layer of the intestine, resulting in lower blood sugar levels (Khan et al., 2019; Tiwari et al., 2014). It has been observed to boost the activity of enzymes involved in the use of glucose in insulin-dependent pathways including hexokinase, glycogen synthetase, glyceraldehydes 3-phosphate dehydrogenase, and glucose 6-phosphate dehydrogenase. On the other hand, the action of insulin-independent enzymes such as glycogen phosphorylase, gluconeogenic enzymes, glucose 6-phosphatase, fructose 1,6- diphosphatase, and sorbitol dehydrogenase is reduced (Khan et al., 2019). In addition, it enhances the regeneration of islets cells (Laha & Paul, 2019). Gymnemic acid's hypoglycemic impact is the result of a series of processes that begin with the modification of incretin, an enzyme that stimulates a decrease in blood glucose level and prompts insulin secretion and release. This mechanism reduces glucose and fatty acid absorption in the small intestine and impairs the capacity of mouth and gut receptors to detect sweetness (Tiwari et al., 2014). Gurmarin works in similar patterns, affecting the tongue's capacity to distinguish between sweet and bitter tastes, therefore, reducing sweet carving (Bhatt & Khobra, 2019; Laha & Paul, 2019). Furthermore, active ingredients conduritol-A, gymnemagenin, and oleanane saponins are found to have hypoglycemic activity. Gymnestrogenin is also known to prevent the absorption of glucose. Gymnemasides are documented to have inhibitory effects on elevated serum glucose levels (Laha & Paul, 2019). In a study by Bhushan et al., (2010), *G. sylvestre* was listed as one of the plants with anti-diabetic activity. The plant is also used in the composition of commercially available polyherbal preparations for diabetic therapy (Bais & Choudhary, 2019). The R&D Department of Hamdard Laboratories (Waqf) Bangladesh developed a polyherbal Unani formulation for diagnosis and prevention of diabetes which is called by the name 'Capsule Gudmur'. The Formulation had a substantial hypoglycemic action in the Glucose Tolerance Test (GTT), which considerably reduced the blood glucose levels of the treated mice at doses of 100 mg and 200 mg/Kg body weight. The impact was equivalent to that of Metformin Hydrochloride, a common oral hypoglycemic medication (Afroze et al., 2012).

4.2 Immunomodulatory activity

Among vertebrates, the Immune system is an intricate defense system that helps to protect them from foreign agents which invade the Immunity of the body (Nagoba & Davane, 2018). Various molecules and cells are generated as a result of the defense mechanism which eliminates the invading foreign and infelicitous agents. The ability of the body to recognize and hold out against microorganism that is capable of detrimental effects is referred to as Immunity of the body. This ability strengthens the body by fighting off and averting diseases that are infectious and retard the damages of tissues and organs (Saroj et al., 2012). Pathophysiology of diseases is said to be responsible for dysfunction related to the Immune system. For years, scientists are trying to initiate efforts to modulate immune responses, and this can be achieved by following two paths which include: the suppression of the reactions which are undesired to the immune system itself or by stimulation of the immune system (Singh et al., 2015). Additional efforts have been made to make immunomodulatory therapy a known alternative for chemotherapy of numerous conditions of diseases, the main focus being the activation of the host's defense mechanism when the immune system shows impaired responses (Behl et al., 2021).

Substances that are said to modify the immune system responses when it faces threat coming towards it are called immunomodulators. These substances possess the ability to modulate, suppress or stimulate both the innate and adaptive arms of the immune system (Nagoba & Davane, 2018)

There is evidence documented by Singh et al., (2015) of a close relation between endocrine and immune system, lymphocyte induced apoptosis that is seen in case of diabetes, cell-mediated and humoral immunity depression and activity of medicinal plants which are related to anti-diabetic properties and this led to the creation of assumptions for endocrine correlation of fat and glucose metabolism as a modulated immune function for extracts of *G. sylvestre* leaf. Thus, in this paper, the immunomodulatory activity of the methanolic extracts of the leaf of *G. sylvestre* on responses to peritoneal macrophage are discussed and the mechanisms have been proved to have potential effects by *G. sylvestre* leaf extract. In vitro studies in a rat model by which immunomodulatory effects by *G. sylvestre* leaf extract are mediated through included the following tests:

Table 8: Immunomodulation Potential of G. sylvestre

Name of tests	**The objective of test conduction**	**Effects by *G. sylvestre* (Singh et al., 2015)**	**Explanation**
NBT assay of reduction in rat peritoneal macrophages	The NBT test evaluates macrophages' ability to degrade	There has been evidence of *G. sylvestre* enhancing the NBT reduction and this	In presence of *G. sylvestre*, macrophage produced more reactive oxygen

	the yellow soluble redox dye NBT to produce blue formazan. (Singh et al., 2015)	happened especially at the concentration level of 50/100/200 μg/ml concentration. After the incubation of macrophages happened in presence of PMA and LPS, an increased reduction of dye (still insignificant) was observed	species (ROS) which indicates that macrophages engaged in increased phagocytosis. Thus *G. sylvestre* has a stimulatory effect on the immunity system.

(Table-8 continued)

Nitrate release test in rat peritoneal macrophages	Nitrate concentration substituted in-vitro gives an estimation of reactive nitrogen intermediate. Nitrogen	Nitrate release test was carried out to microphage culture and as stimulation of nitrate release is concentration-dependent manner,	This gives evidence that *G. sylvestre* has a stimulatory effect on the immunity system.

	intermediates are important to detect the anti-tumor and anti-microbial activity of macrophages present in rodents as well as human macrophages. (Padgeit & Pruett, 2017; Singh et al., 2015)	there was significant stimulation seen in case of treatment with *G. sylvestre* extract. A higher level of stimulation was observed when the macrophage culture containing *G. sylvestre* extract was kept in presence of both PMA and LPS	

The immune activities related to the *G. sylvestre* extracts in rat macrophage revealed that they have a key role in the innate immune system as macrophages perform major functions such as phagocytosis of pathogens, production of de-novo ephemeral, reactive oxygen species (ROS). The oxygen-dependent defense mechanism of the innate immune system involves the release of reactive oxygen species by respiratory oxygen burst and this stimulation can be brought out by stimulation with components such as LPS (lipopolysaccharide), PMA (phorbal myristate acetate), and these two compounds were used as standards in this experiment. For the case of NBT test, the macrophage's ability to reduce the yellow redox dye to a blue-colored formazan formation is measured and the degree of production of formazan provides an estimated superoxide anion measure. Also, nitric oxide is used for the estimation of cytotoxicity regarding the macrophages. (Singh et al., 2015).

Moreover, Malik et al., (2009) examined the aqueous extract from *G. sylvestre* leaves by an evaluation of the Neutrophil locomotion and chemotaxis, phagocytosis of Candida albicans, and Nitroblue tetrazolium tests on the immunomodulatory action. The phagocytic activity of human neutrophils was dramatically boosted by the extract of *G. sylvestre*, as compared to control showing the potential immune-stimulating activity.

4.3 Anti-oxidant activity

An antioxidant is a word that signifies "against oxidation." An antioxidant is a chemical that, at low concentrations relative to those of an oxidizable substrate, considerably slows or inhibits the oxidation of that substrate. (Sehwag & Das, 2014; Yadav et al., 2016). They have a variety of physiological functions in the body (Kaskoos et al., 2015). Antioxidants are essential for protecting human health and protecting the quality of food (Sehwag & Das, 2014). They are our body's first line of defense against free radical-induced damage as oxidation in a biological cell system can cause cell damage or cell death. Increased oxidative stress on the cellular structure is caused by uncontrolled free radical generation, which leads to alterations in molecular pathways that drive the pathogenesis of various major illnesses, including cancer, and physiological aging. Thus antioxidants are important for good health and well-being (Behera, 2019; Sehwag & Das, 2014; Yadav et al., 2016). The plant material's antioxidant components serve as radical scavengers, assisting in the conversion of radicals to less reactive forms (Yadav et al., 2016). As antioxidants reduce the oxidative stress in cells they are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases, diabetes, and inflammatory diseases (Kaskoos et al., 2015).

The methanol extract of *G. sylvestre* leaves has potent antioxidant properties (Behera, 2019). Gunasekaran et al., (2019) investigated its antioxidant activity using three distinct methods: DPPH free radical quenching activity, reducing power test, and hydroxyl free radical quenching activity. Using dried leaf extract, they noticed strong antioxidant activity in all three assays. Phosphomolybdenum assay and superoxide anion radical scavenging activity test was also carried out for detecting this activity (Behera, 2019). Methanolic extracts were used in both studies (Behera, 2019; Gunasekaran et al., 2019)

2, 2-diphenyl-1-picrylhydrazyl (DPPH) has a stable free radical which can be reduced by taking hydrogen or electrons from other molecules. In solution, the DPPH radical displays a deep violet appearance with a 520 nm absorption band. It becomes colorless and pale when neutralized, and the shift in optical absorption enables for evaluation of radical scavenging activity. In the reducing power test, the conversion of iron in ferric chloride from ferric to ferrous form is calculated. On the other hand, in the hydroxyl free radical quenching activity test, hydrogen peroxide was mixed with phosphate buffer saline and the absorbance of the mixture was noted by using a UV-visible spectrophotometer against a mixture of extract and Phosphate buffer saline (Gunasekaran et al., 2019). Phosphomolybdenum assay depends on the extract reducing Phosphate-Molybdenum (VI) to Phosphate-Molybdenum (V) and then forming a green phosphate complex at an acid pH. In this assay, the total antioxidant potential of the methanol extract of *G. sylvestre* was calculated by ascorbic acid equivalents (AAE) per gram. In the case of the superoxide anion radical scavenging activity test, under aerobic conditions, nitro blue tetrazolium (NBT) was reduced in the presence of nicotinamide adenine dinucleotide (NADH) and phenazine methosulfate (PMS). The absorbance of the mixture was read at 560 nm (Behera, 2019). The results of the study are given in the table.

Table 9: Antioxidant potential of Methanolic extract of G. sylvestre.

Name of method	**Standard used**	**Result**	**References**
DPPH free radical quenching activity	Ascorbic acid	The activity of the methanolic extract was 82.5 percent at its maximum concentration of 6 µg.	(Gunasekaran et al., 2019)
Reducing power test	Ascorbic acid	Increasing concentration of extract showed increased absorbance that indicates an increase in the reducing activity	(Gunasekaran et al., 2019)
hydroxyl free radical quenching activity	Rutin	200 µg concentration of extract exhibited 59.8% inhibition	(Gunasekaran et al., 2019)
Phosphomolybdenum assay	Ascorbic acid	the highest total antioxidant capacity was observed with a value of 164.15 0.14 mg AAE/g.	(Behera, 2019)

(Table-9 continued)

superoxide anion radical scavenging activity	Ascorbic acid	At 500g/ml concentration, a methanol extract of *G. sylvestre* scavenges up to 56.250 % of superoxide radicals, whereas normal ascorbic acid scavenges 71.875 percent.	(Behera, 2019)

This plant was discovered to have greater DPPH radical scavenging than butylated hydroxyl toluene (BHT) and to minimize LDL oxidation in another investigation. (Khan et al., 2019). When the antioxidant activity of *G. sylvestre*, *Enicostemma littoral*, *Momordica charantia*, and their composite extract was compared, the study revealed that the antioxidant activity of all the mentioned plants was concentration-dependent. *G. sylvestre* exhibited higher antioxidant potential, ranging from 3.92 to 72.22 percent for 7.81 to 1000 g/ml, in comparison with *E. littorale* that has a ranging of 2.61 to 69.08% for 7.81 to 1000 μg/ml. However, the extract of *G. sylvestre* had less antioxidant capacity than *M. charantia* (Kaskoos et al., 2015). This plant holds many antioxidants as active ingredients like Terpenoid, Flavonoid, Cinnamic acid, Folic acid, Tannin, Phenol, Ascorbic acid, etc. (Laha & Paul, 2019). Because humans lack the enzyme that catalyzes the last stage of the biosynthetic pathway, ascorbic acid has taken on the role of vitamin for them.

Therefore, ascorbic acid is marked as a crucial water-soluble antioxidant (Mandl et al., 2009). The antioxidant activities of β-carotene have been related to lowering the risk of certain illnesses (Milani et al., 2017). Many tannin molecules have also been shown to have antioxidative properties which the plant is rich in (Chung et al., 2016).

Table 10: Table of antioxidants of G. sylvestre with their mechanism (Laha & Paul, 2019)

Antioxidant	Mechanism
Ascorbic acid	Neutralize hydroxyl, superoxide, and hydrogen peroxide radicals
anthraquinone, flavones, flavonoids like epicatechin, Apigenin, Luteolin, Kampferol, Hentriacontane, Pentriacontane, Phytin, Resin, Lupeol, β-amyrene related glycosides, an alkaloid (conduritol), α and β- chlorophyll, Stigmasterol, d-quercitol, Nonacosane, Lignin etc.	Scavenging free radicals
Terpenoid, Flavonoid, Cinnamic acid, Folic acid, Tannin, Phenol.	Scavenging SO2, H2O2, and having reducing power ability.

4.4 Antiobesity

Obesity is a major predisposing factor that roots different kinds of diseases. World health organization has shown that obesity was two-fold increased since the year of 1980s and follow up study in 2014 showed that as much as 1.9 billion people were suffering from being overweight and the number of obese people was estimated to be 600 million (Global Status Report On Noncommunicable Diseases 2014). Health problems such as type 2 diabetes, hypertension, cardiovascular disease, asthma, osteoarthritis, and in the worst case, various types of cancers may arise due to problems related to obesity. Increased attention therefore should be given to obesity-associated problems to maintain public health. The major causes of obesity are the imbalance of energy due to expenditure and consumption of calories, the rise of high-fat diet intake. The rise of high-fat diet alone can end up causing disorders related to metabolic function including adipocyte hypertrophy, insulin resistance, and chronic adipose inflammation (Kumar et al., 2012; Pothuraju et al., 2013) There has been a long search for adequate herb or plants to reduce obesity-associated problems and *G. sylvestre* has shown positive results for lowering this notorious predisposing factor.

A study conducted on 4 groups of Wister rats, where each group contained several 8 rats each showed a significant reduction of body mass index (BMI) after close monitoring of 4 weeks when treated with *G. sylvestre* extract. All groups were treated for 28 days, and group 1 had normal healthy Wister rats, group 2 had pathogenic control group rats which were fed with high-fat diet (HFD), group 3 rats were those who were fed high-fat diet and then treated with *G. sylvestre* extract and finally, group 4 contained rats which were treated with rimonabant from the 8th day (for 21 days) after being fed high-fat diet. The blood pressure including systolic, diastolic, and mean arterial pressure using the tail cuff of rat method were also measured (Kumar et al., 2013).

Table 11: The following effects were perceived for the target group or G. sylvestre extract-treated group (Group 3) (Kumar et al., 2013).

Effects exerted:	Result due to the use of a water-soluble fraction of ethanolic *G. sylvestre* extraction on the groups:
Effects on apolipoprotein A and B, insulin, glucose, serum leptin.	Serum leptin, LDH, Apolipoprotein B, and glucose were seen to be decreased, and an increase in Apolipoprotein A was observed in the study for group 3 or ethanolic extract-treated group.
Serum profile for lipids	Components such as total cholesterol, low density, and high-density lipoprotein, triglyceride, very low-density lipoprotein cholesterol were shown to be decreased for the group 3 Wister rats whereas the HFD group and control group showed an increase.
Effects on Na+ and K+ ATP channels present on liver and heart and on lipid peroxidation	Lipid peroxide levels were expressed with Thiobarbituric acid reactive substances (TBARS) level and decreased in the case of group 3. The Na+/K+ ATPase levels in the liver and heart increased significantly.

(Table-11 continued)

Effects on the level of hepatic cholesterol, visceral and organ fat pad weights	**The visceral fats present in perirenal, mesenteric, and epididymal fat pad weights were seen to be reduced in the group 3 rats that were treated with *G. sylvestre* extract.**
Effects are seen on histopathology	There were some changes visible along with congestion which was limited to normal range when photomicrograph of heart tissue of group 3 rats was analysed for fatty and morphological changes. No fat accumulation was observed for the treated group with *G. sylvestre* extract.

4.5 Antimicrobial activity

Foodborne illness is a prevalent food safety issue produced by the ingestion of contaminated food items, and it has long been a major public health concern (Azziz-baumgartner et al., 2005; Kirk et al., 2017). Moreover, antibiotic resistance in bacteria is a viable challenge in today's society. Plant-derived biomolecules appear to be one of the most promising options for dealing with antibiotic-resistant human and plant microorganisms (Vibala & Praseetha, 2019).

Thanwar & Dwivedi, (2019) conducted a study in which the antibacterial activity of a hydro-alcoholic extract of *G. sylvestre* leaves was investigated. Gram-negative *Escherichia coli*, gram-negative *Pseudomonas aeruginosa*, and gram-positive *Staphylococcus aureus* were used as test organisms. Using the agar well diffusion method, antibacterial activity was assessed and broth dilution technique was used to determine the minimum inhibitory concentration (MIC). The findings of this study give scientific support for *G. sylvestre*'s traditional usage as an antibacterial agent. Based on the results of the investigation, Thanwar & Dwivedi concluded that *G. sylvestre* may have the most potential for treating infectious disorders caused by the bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

Table 12: Antimicrobial activity of hydroalcoholic leaf extract of G. sylvestre against microorganisms (Thanwar & Dwivedi, 2019).

Zone of inhibition with different concentration µg/ml				
S. No.	**25%**	**50%**	**75%**	**100%**
E. coli	10mm	11mm	12mm	15mm
Pseudomonas	10mm	10mm	13mm	13mm
S. aureus	6mm	10mm	12mm	13mm

In another study, Vibala & Praseetha, (2019) used three Gram-positive bacterial strains which are *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa*, and two gram-negative stains of *Streptococcus and Staphylococcus aureus* to check the antibacterial activity of ethyl acetate extracts of *G. sylvestre*. The test was carried in vitro using the agar disc diffusion technique in the presence of bacteria. The results of the investigation were compared with the antibiotic Ampicillin.

Table 13: Antimicrobial activity of ethyl acetate extracts of G. sylvestre (Vibala & Praseetha, 2019)

Organisms	**Zone of Inhibition (mm)**			**Antibiotic (1mg/ml)**
	Concentration (µg)			
	1000	**750**	**500**	
Streptococcus	20	17	11	47
Staphylococcus aureus	10	9	9	28
Escheria coli	13	9	9	25
Salmonella typhi	14	10	10	29
Pseudomonas aeruginosa	10	8	8	30

The remarkable antifungal efficacy of the plant was also discovered by Gunasekaran et al., (2019). They inspected the potency of *G. sylvestre* methanol extract against *Fusarium oxysporum* and found no colonies after three days of incubation at the maximum dose of 4 mg/ml.

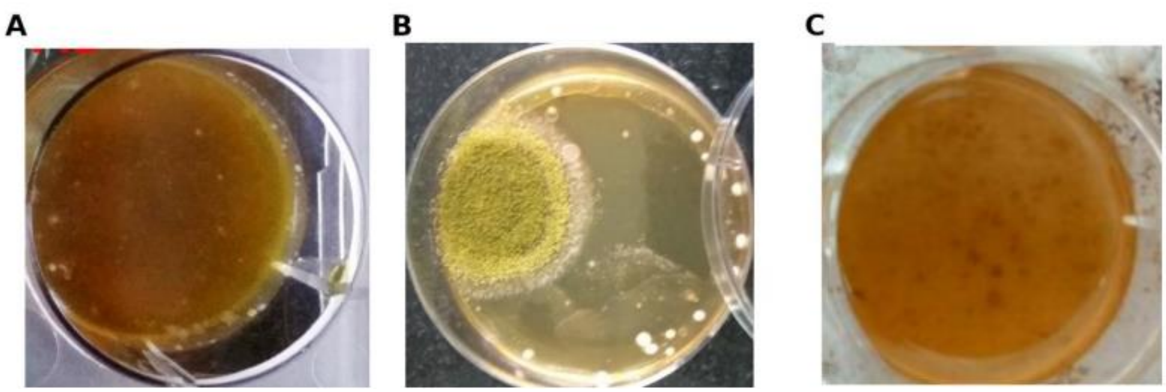


Figure 11: Antifungal activity of G. sylvestre (Gunasekaran et al., 2019).

The figures above indicate that in the greatest concentration of *G. sylvestre* extract, there is no fungal colony at all, using methanol as a negative control and gold chloride as a positive control. A – *G. sylvestre* Methanol extract – 4 mg/ml. B – Control negative (methanol), C- control positive (Gold chloride). Dash et al., (2018) documented that for fungal species such as *Aspergillus niger*, *G. sylvestre* fruit extract demonstrates inhibitory zones to be 10.75 mm and the root extract of *G. sylvestre* demonstrates a zone of inhibition of 11.33 mm with 100 mg/ml.

According to Porchezhian & Dobriyal, (2003), this plant is also known to possess antiviral activity. Gymnemic acids A and B isolated from the plant are held responsible for this potential. Gymnemic acid A had the greatest effectiveness against the influenza virus at a dosage of 75 mg/kg body weight, followed by gymnemic acid B. However, some leaf fractions lack this activity. Overall antimicrobial action of the plant extract may be attributed to the presence of alkaloids, phenols, and terpenoids in the extract (Renganathan et al., 2017).

4.6 Anti-inflammatory activity

Inflammation is a protection approach that has developed in higher species in response to harmful stimuli such as microbial infections, tissue injuries, and other harmful circumstances. It is a critical immunological response (Ahmed, 2011). Inflammation is defined as a preventive strategy done by the body to eliminate harmful impulses and repair damaged tissue (Cássia et al., 2013). As a result, acute inflammation has been classified as a component of innate immunity, the host's first line of defense against external invaders and dangerous chemicals (Ahmed, 2011). However, if the inflammation is allowed to continue uncontrolled, it can lead to health disorders like autoimmune or auto-inflammatory, neurological problems, or cancer (Dinarello, 2010). To resolve this, a useful therapeutic option for diseases might be the use of anti-inflammatory drugs (Ahmed, 2011). For this reason, medicinal plants and their isolated substances in folk medicine are used globally to treat various inflammatory disorders, such as inflammation of the lungs and skin (Cássia et al., 2013).

Tiwari et al., (2014) conducted an investigation in which carrageenan-induced paw edema and cotton pellet induced granuloma rats were taken and anti-inflammatory action in rats were studied with leaf extracts of *G. sylvestre* at measures of 200, 300, and 500 mg/kg. It was reported that the plant extract lowered the volume of the paw edema considerably at concentrations of 300 mg/kg by 48.5% within 4 hours, while the paw edema dropped by 57.6% when drug phenylbutazone was used. Moreover, in comparison to the control group, the aqueous extract at a concentration of 200 and 300 mg/kg showed a decrease in the granuloma. In another study, run by Diwan et al., (1995), carrageenan-induced rat paw edema and peritoneal ascites in mice were examined incorporating *G. sylvestre* extract. Highest inhibition was demonstrated to be 42.95 ±1.5% at extract concentration of 400 mg/kg compared to naproxen 67.34 ±7.3% at 100 mg/kg.

Table 14: Effect of G. sylvestre in the carrageenan-edema model (Diwan et al., 1995)

Dose of gymnema extract mg/kg	**% of Inhibition**
200	22.22 ±2.5
400	42.95 ±1.5
600	45.20 ±5.5%

When compared to controls, ascitic fluid volume was inhibited by 62.50 ±4.5 percent in plant extract and 64.38 ±7.4 percent in naproxen-treated animals. Tannins and saponins, which are bioactive ingredients in *G. sylvestre*, are responsible for the plant's anti-inflammatory effect (Tiwari et al., 2014).

4.7 Anti-hyperlipidemic activity

As many as 17 million death is estimated to occur worldwide due to hyperlipidemia alone (Thomas et al., 2018). Hyperlipidaemia also plays a major role in coronary heart diseases (CHD) and atherosclerosis. Due to lipid peroxidation, endothelial damage can occur and thus chronic diseases like atherosclerosis can arise. The resulting endothelial damage can initiate the permeation through which LDL can reach to intima layer and this results in atherosclerotic damage and oxidation (Li et al., 2014; Vogiatzi et al., 2008). Hyperlipidemia itself is known as a family of disorders and is distinguished by an increased level of fats or lipid in the blood. Meanwhile, many metabolic processes include the regulation of fat, but an excess amount of fat may cause disorders such as coronary heart disease. In the coronary epidemiological study of 2002, a positive correlation between hyperlipidemia, level of blood lipids, and complications of CHD (Marquis, 1983). In the case of hyperlipidemia, triglycerides carrying lipoproteins and the amount of cholesterol in plasma surpasses the normal levels (DiStefano, 1986).

G. sylvestre is known for the potential regulation of glucose levels in the blood and it is also a renowned folk medicine that is used to reduce cholesterol levels of blood besides the homeostasis related to the regulation of blood sugar levels. It is currently found its usage in herbal preparation such as tablets, tea bags, confectionery, and supplements (Tiwari et al., 2014). A phytochemical study conducted in Nigeria which included treatment of Sprague Dawley rats with methanolic extract of *G. sylvestre* leaves showed a significant reduction in blood glucose level and presented noticeable hyperglycaemic ameliorative effects on dyslipidemia that was induced through medication (Mann & Babalola, 2019). The possible anti-diabetic and hypoglycaemic effects were speculated to be mediated through the presence of phytochemical compounds such as flavonoids, terpene, alkaloids, and saponins in the leaf extracts of *G. sylvestre* (Okokon et al., 2006). In a study conducted by D. K. Singh et al., (2017) on more whist rats with *G. sylvestre* powdered extract as test drug and standard drug as atorvastatin also revealed potential hypolipidemic effects of it. The 30 rats were divided into a group of 5 and were fed a high-fat diet. After four weeks of diet, the treatment regimen started. The following hypoglycaemic effects were observed:

Table 15: Hypoglycaemic effects of G. sylvestre (Singh et al., 2017).

Name of mediated effects:	**Result of the G. sylvestre treated group:**
Mediated effects of body weight	The body weight was reduced in the case of both the *G. sylvestre* treated group which included treatment with 100 and 200 mg/kg *G. sylvestre* extract.
Mediated effects of total cholesterol (TC)	The highest reduction of total cholesterol was noticed in the case of the atorvastatin treated group followed by groups that were treated with 200 and 100 and mg/kg *G. sylvestre* extract respectively.

(Table-15 continued)

Mediated effects of triglyceride (TG)	**Reduction of triglyceride levels in the atorvastatin treated group was shown to have the most decreased level of triglyceride followed by 200 and 100 mg/kg *G. sylvestre* extract-treated group respectively.**
High-density lipoprotein cholesterol (HDL-C)	The same result as previous effects TC and triglyceride observed for reduction of HDL-C for atorvastatin group being the most reduced followed by 200 and 100 mg/kg *G. sylvestre* extract-treated group.
Effects of Very low-density lipoprotein (VLDL) and Low-density lipoprotein (LDL)	Both atorvastatin group and drug-treated group with 200 and 100 mg/kg *G. sylvestre* extract showed a potential reduction of VLDL and LDL cholesterol.

Chapter 5

Discussion

Medicinal plants are regarded as a wealth of resources for substances that may be utilized in medicine development either pharmacopoeial, non-pharmacopeia or synthetic pharmaceuticals. No state or culture can disregard the potential of medicinal plants even when they are highly wealthy in alternative and synthetic therapeutic products. In addition, these plants play a key role in the evolution of human civilizations across the world in which several plants are regarded as vital nutritional sources and are thus suggested for their medicinal properties. *G. sylvestre* is one of these therapeutically essential plants containing vital physiologically important compounds. It contains saponins, flavonol, glycosides and gymnemanol. (Table-4 and Figure 8). These active ingredients exhibit several requisites for biological activities which is displayed in table-7 of this review. This plant also holds a variety of necessary minerals (Table-6). *G. sylvestre* is a plant well recognized in the Indian pharmacological code and is well known in traditional Indian medical systems such as Sidha, Unani, and Ayurveda. This plant is a treasure of numerous pharmacological characteristics and excellent anti-diabetic potential. We live in a world where diabetes mellitus is increasingly common and is estimated to be a serious public health problem by 2030. In these circumstances, with a wide spectrum of medicinal properties show an efficient cure for diabetes. The flavor of this plant is shown to reduce the capacity to detect sweet flavors. It is one of the most significant anti-diabetic therapeutic plants. It has anti-diabetic action in both experimental and clinical studies, and it raises our insulin levels (Chapter-4.1). Other than this, the plant is observed to have potential antioxidant, antimicrobial, anti-obesity, anti-hyperlipidemic, anti-inflammatory, and also immunomodulatory activities. The medicinal potential of this plant has gained a lot of attention, especially because of its anti-diabetic properties, which have been discovered by various studies. I believe that 'chapter 4' of this review contains a lot of evidence to support this declaration. Therefore, this plant can be a good alternative for many diseases and disorders.

Chapter 6

Future perspective

According to reports, nearly 80% of people in underdeveloped nations rely on natural remedies to treat ailments and address their major health issues. Despite their high demand and medicinal potential, only small percentage of the plants have been studied for their therapeutic potential. *G. sylvestre* is one of the therapeutically essential plants that contains significant physiologically important compounds. Flavonoids, alkaloids, terpenoids and tannins are chemicals found in plants that have life-saving medicinal properties. Anti-metastatic, anti-diabetic, hypoglycemia, antioxidant, hepatoprotective, anti-inflammation, anti-bacterial, anti-fungal, anti-viral are among the actions that the plant stands potential for. Other than these, because the plant has been shown to regenerate beta cells and increase insulin secretion, it can also be utilized as a PCOS (polycystic ovary syndrome) treatment. Lastly, the bioactive compounds of *G. sylvestre* has also found to be an effective medication for COVID-19, by blocking the main protease enzyme called 3CLpro which is essential for viral replication. Thus, this plant is being mismanaged rather than being utilized to its full potential. To avoid this plant from going to waste, appropriate production of therapeutic preparations from it should be ensured, and the plant's long-term use should be closely regulated. In addition to these, people should also be made aware of the proper use of the plant so that they can get maximum benefit from this plant.

Chapter 7

Conclusion

A severe hazard to mankind in the developing trend is the increased incidence of illnesses and related consequences with commercial medicaments. To avoid this, naturopathic therapy is an efficient alternative that provides relief from the enormous expense of costly medicines and is relatively safe with fewer adverse effects. *G. sylvestre* is one of these therapeutically essential plants that contains significant physiologically important compounds and accounts for numerous pharmacological properties. There are many assays performed with this plant to evaluate its potentiality as an antioxidant, antimicrobial, anti-obesity, anti-hyperlipidemic, anti-inflammatory, and also as immunomodulatory agent but regrettably the inspections are with a higher number of in-vivo assays in only animals. Any trail in humans for these various essential biological functions are scarcely found. Although data on anti-diabetic action is available, its impact on the cardiovascular system has not been well investigated in both preclinical and clinical context and so this plant is not found to be used as a commercial anti-diabetic treatment. Thus, I want to direct future researchers to screen this plant more as a basis for developing novel and effective treatments that the plant stands potential for. Also, there are presently no defined methods in place to ensure the quality and efficacy of its product. From a pharmacological standpoint, safety is a relative term that needs to be clarified by more studies in this area. A significant limitation is the absence of standards for herbal compositions. Although there is a wide range of plant resources accessible, only a handful have been experimentally confirmed and scientifically authorized as illness treatments. Therefore, the principal motive of this study is to convey the significance of this plant as the findings of the assays appeared to be very promising.

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