In vitro antidiabetic activity and *in silico* molecular docking of Nilavembu, *Andrographis paniculata* (Burm.f) Nees

The thesis submitted in partial fulfillment of the Degree of Master of Philosophy (M.Phil.)

By

Deepika, E.

(Reg. No. 19MPZOF001)

Department of Zoology

Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore- 641043

DECEMBER 2020

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21.12.2020

Signature of the Head of the Department

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Signature of the Supervisor

Certificate

CERTIFICATE

This is to certify that the dissertation entitled "***In vitro* antidiabetic activity and *in silico* molecular docking of Nilavembu, *Andrographis paniculata* (Burm.f) Nees**" submitted by **Deepika, E** for the degree of Master of Philosophy (M. Phil.) is the record of work carried out by her during the period from July 2019 to December 2020 under the guidance of Dr. K.S. Santhy, Professor, Department of Zoology and this work has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship, Titles in this University or any other University or other similar institution of Higher learning.

21.12.2020

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Signature of the Supervisor

Declaration

DECLARATION

I declare that the dissertation entitled "***In vitro* antidiabetic activity and *in silico* molecular docking of Nilavembu, *Andrographis paniculata* (Burm.f) Nees**" submitted by me for the degree of Master of Philosophy (M. Phil.) is the record of work carried out by me during the period from July 2019 to December 2020 under the guidance of Dr. K.S. Santhy, Professor, Department of Zoology and has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship, Titles in this University or any other University or other similar institution of Higher learning.

E. Dhanjil

Signature of the candidate

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Deepika E

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LIST OF ABBREVIATIONS

ADMET	-	Absorption, Distribution, Metabolism, Excretion and Toxicity
CAS	-	Chemical Abstract Service
DMSO	-	Dimethyl sulfoxide
DNA	-	Deoxyribo Nucleic Acid
DNMT1	-	DNA methyl transferase1
DPPH	-	1, 1'-diphenyl-2-picryl hydrazyl hydrate
GC-MS	-	Gas Chromatography-Mass spectrometry
GLP-1	-	Gulcagon Like Peptide-1
HBA	-	Hydrogen Bond Acceptor
HBD	-	Hydrogen Bond Donor
HSD1	-	Hydroxysteroid Dehydrogenase type I
IL-2	-	Interleukin-2
MD	-	Molecular Dynamic
MW	-	Molecular Weight
NIDDM	-	Non-Insulin Dependent Diabetes Mellitus
NFAT	-	Nuclear Factor Activated T cells
PDB	-	Protein Data Bank
ROS	-	Reactive Oxygen Species
TCA	-	Trichloro acetic acid
TCM	-	Traditional Chinese Medicine
TM	-	Traditional Medicine
UV-Vis	-	UltraViolet Visible spectrometry
WHO	-	World Health Organization
PTP1B	-	Protein-tyrosine phosphatase 1B

Introduction

1

INTRODUCTION

Herbs are staging a comeback and a relook for herbal therapy and natural products is happening all over the globe. The herbal products had been priced for their medicinal, flavouring and aromatic qualities for centuries. In contrast, the synthetic products of the modern age surpassed their importance, for a while. However, the synthetic therapeutic products could not be accepted more due to severe side effects. Now, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Natural products were found as storehouse of different molecular designs, even beyond the human imagination (Gilani, 2005).

Most synthetic drugs produced by imitating the herbal medicines, but they are created artificially in pharmaceutical laboratories. At least one third of all used products have plant origin. The use of plants as natural, safe, accessible and inexpensive materials, compared to synthetic antibiotics, has been growing for the treatment of bacterial infections. Also, herbal medicines have more popularity among the people compared with chemical ones. Plants used by indigenous peoples are believed to be the starting point for developing critical modern drugs like aspirin, digoxin, tubocurarine, morphine, atropine, reserpine and quinine (Ghani *et al*., 2014).

Medicinal herbs are a rich source of bioactive substances, antioxidants, flavonoids, and phenolic substances and have multiple health effects. Constant and renewed public interest on alternative and complementary medicine lies mainly due to the high cost of new drugs, increased side effects, microbial resistance and lack of curative treatment for several chronic diseases. Furthermore, the usage of herbal extracts or active compounds (such as chlorogenic acid, ferulic acid, cinnamic, rosmarinic acids) in food, cosmetic and pharmaceutical industries have been increased in the last years, so that the biological and phytochemical study of medicinal plants is essential and an exciting area of research (Gohari *et al*., 2011; Bonarska *et al*., 2011; Sytar *et al.*, 2012; John *et al*., 2015).

Ayurveda a pillar of Indian traditional system of medicine concentrate more in potentiality of many medicinal plants. Thereby scientists and researchers employed a plenty of plants in findings of new drug where by adopting various strategies leads to the discovery. A kind of

approach is referred to as arbitrary screening in which plants from a particular country or region are identified and then made to work through several testing systems in search for new compounds that might take to the new drug discovery (Foster, 2010). Even though orthodox medicinal healers are being using the medicinal plants in treating illness for millions of years, there has always been a persisting question in biological circles about their therapeutic potency. As an outcome, many studies have been done relating to their pharmacological activity, but immense majority of plants endure to be studied for their chemical components and medicinal effects. World health organization (WHO, 2002) revealed a record of 21000 important medicinal plants.

Diabetes Mellitus is a chronic metabolic disorder that affects human body in terms of physical, psychological and social health. It is defined as a group of disorders characterized by hyperglycaemia, altered metabolism of lipids, carbohydrates and proteins (Patel *et al*., 2012a and Warjeet, 2011). Inappropriate hyperglycaemia is caused by a relative or absolute deficiency of insulin or by resistance to the action of insulin at the cellular level (Revathi *et al*., 2014)

Diabetes is a complex, chronic illness requiring continuous medical care with multifactorial risk-reduction strategies beyond glycaemic control (American Diabetes Association, 2015). Progress in understanding the metabolic staging of diabetes over the past few years has led to significant advances in the regimen for treatment of this devastating disease. Management of diabetes without any side effects is still a challenge for medical system (Patil *et al*., 2011).

The prevalence of Diabetes Mellitus is increasing throughout the world, especially in developing countries, including India due to changing lifestyles of people and genetic background (Dhanwal *et al*., 2014). India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "Diabetes capital of the world" (Das *et al*., 2015). According to International Diabetic Federation, (2013) Type 2 Diabetes is one of the most significant global health problems of modern time; more than 317 million people have been diagnosed with diabetes and about 187 million are living undiagnosed. Recently published Indian Council of Medical Research–India Diabetes (ICMR–INDIAB) national study reported that there are 62.4 million people with Type 2 Diabetes and 77 million people with pre-diabetes in India (Anjana *et al*., 2011).

Herbal treatments have been used in patients with insulin dependent and noninsulin dependent diabetes, diabetic retinopathy, diabetic peripheral neuropathy and other complications of diabetes (Nashte *et al*., 2013). Diabetes has been treated with several medicinal plants or their

extract based on the folklore medicine (Malpani and Manjunath, 2013). The mechanism is most often not completely understood and so more and more studies are being conducted to elucidate the mechanism of action of different plants and natural compounds (Cristina *et al*., 2012).

Andrographis paniculata (*Acanthaceae*) plant is native to China, India and Taiwan. It is a medicinal herb with an extremely bitter taste used to treat liver disorder, bowel complaints of children, colic pain, common cold and upper respiratory tract infections. The aerial part of *Andrographis paniculata* is commonly used in Chinese medicine. According to Chinese medicine theory, *Andrographis paniculata* cools and relieves internal heat, inflammation and pain and hence used for detoxication.

Since ancient times *Andrographis paniculata* is used in traditional siddha and ayurvedic systems of medicines as well as in tribal medicine in India and some other countries for multiple clinical applications. The herb is the well-known drug Kalmegh or 'green chiretta' and forms ingredient of a reputed house hold medicine. Powdered plant mixed with mustard oil is using for the treatment of itching. The macerated leaves and juice together with certain spices prescribed for relief from gripe and other stomach ailments in infants and also used as domestic medicine for flatulence and diarrhoea of children. It is used in torpidity of liver, neuralgia and convalescence after fever. A decoction of the plant is a blood purifier while an infusion is used in fever. A decoction or infusion of the leaves is useful in general debility and dyspepsia. The leaves and root are also used as febrifuge, tonic, stomachic, cholagogue and anthelmintic (Chopra *et al*., 1956 and Nadkarni *et al*., 1954).

Andrographis paniculata or Kalmegh is one of the most widely used plants in ayurvedic formulations. *Andrographis paniculata* was recommended in Charaka Samhita dating to 175 BC for treatment of jaundice along with other plants in multi plant preparations (Hooker 1885 and Sharma, 1983). It has also been used traditionally for sluggish liver as antidote in case of colic dysentery and dyspepsia (Handa and Sharma, 1990). It has been employed with benefit in case of general debility in convalescence after fever, disorders of liver and advanced stages of dysentery (Dastur, 1959). The juice of fresh leaves is a domestic remedy in the treatment of colic pain, loss of appetite, irregular stools and diarrhea (Saxena, 1998). Unlike other species of the genus, *Andrographis paniculata* is of common occurrence in most places in India, including the plains and hilly areas up to 500 m, which accounts for its wide use. Since time immemorial, village and ethnic communities in India have been using this herb for treating a variety of ailments. The

demand of *Andrographis paniculata* is increasing day by day due to its importance in the treatment of different ailments.

The herb contains diterpenoids, flavonoids and polyphenols as the major bioactive components. In comparison with other Chinese medicinal herbs, *Andrographis paniculata* showed a wide variety of health benefits, due to the presence of bioactive compounds. A few derivatives have been semi-synthesized to enhance their bioactivity than original compounds, suggesting its potential for drug development (Matsuda *et al*., 1994).

Andrographis paniculata is an erect annual herb extremely bitter in taste in each and every part of the plant body. The plant is known in north eastern India as maha-tita literally "king of bitters", and known by various vernacular names. It is also known as bhui-neem, since the plant is smaller in size and has a similar appearance as that of Neem (*Azadirachta indica*). In Tamil it is called "sirunangai" or "Siriyanangai". The genus *Andrographis* consists of 28 species of small annual shrubs essentially distributed in tropical Asia. Only a few species are medicinal of which *Andrographis paniculata* is the most popular.

The aerial parts, roots and whole plant of *A. paniculata* have been used for centuries in Asia as traditional medicine for the treatment of various ailments. It has been used by traditional medical practitioners for stomachaches, inflammation, pyrexia, and intermittent fevers (Chopra 1980; Jarukamjorn *et al*., 2010; Chaturvedi *et al*., 1983; Balu *et al*., 1993). The whole plant has been used for several applications such as antidote for snake-bite and poisonous stings of some insects, and to treat dyspepsia, influenza, dysentery, malaria and respiratory infections (Chopra 1980 and Jarukamjorn *et al*., 2010). The leaf extract is a traditional remedy for the treatment of infectious disease, fevercausing diseases, colic pain, and loss of appetite, irregular stools and diarrhea (Saxena *et al*., 1998).

In Malaysia, a decoction of the aerial parts is used to treat common cold, hypertension, diabetes, cancer; malaria and snake bite (Perry 1980). It is an important constituent of at least 26 Ayurvedic formulas in Indian pharmacopoeia. In traditional Chinese medicine, it is seen as the cold-property herb used to rid the body of heat and fever and to dispel toxins from the body (Deng 1978). In Ayurvedic medicinal system, tribals of Tamilnadu, India uses this herb for a variety of ailments like dysmenorrhoea, leucorrhoea, pre-natal and post-natal care, complicated diseases such malaria, jaundice and gonorrhea.

The evidence collected till now shows immense potential of medicinal plants used in traditional systems. The herb, *Andrographis paniculata* is the main source of the bitter principle. The extremely bitter and characteristic taste of *A. paniculata* of the *Acanthaceae* family, gives it the term "kings of bitters". Several recent studies have validated some of the medicinal properties of this plant and its use in traditional medicine; such properties include its antimicrobial activity (Singha *et al*., 2003) hepatoprotective capacity (Trivadi and Rawal, 2001), antimalarial activity (Rahman *et al*., 1999) and ant diarrhoeal potential (Gupta *et al*., 1993)

Use of *Andrographis paniculata* as a natural herb in India is very common. Crude drug consists of dried or fresh leaves or the aerial portion of the plant. Sometimes the whole plant including the roots is used. Panchang (stem, leaves, flowers, root and seeds) of the plant is being used in various formulation of Indian system of medicine for the treatment of many diseases. The drug normally should not contain more than 2 % of foreign organic matter (Chopra *et al*., 1956).

The market potential of *A. paniculata* is very high (Sharma *et al*., 2008), it is highly consumed as stomachic (Agrawal *et al*., 2005), hepatoprotective (Agrawal *et al*., 2005), dyspepsia (Agrawal *et al*., 2005), anthelmintic (Agrawal *et al*., 2005), bitter tonic, (Kandya, 2005), febrifuge, (Kandya 2005). With reference to trade an estimated consumption of *Andrographis paniculata* aerial parts is 250 tones (Sharma *et al*., 2008). Important, biologically active plant metabolites isolated from various parts of this plant are andrographolide, 14deoxy-11-oxoandrographolide, 14-deoxy-11, 12 didehydroandrographolide and neoandrographolide (Balmain and Connolly, 1973). The other important compounds isolated from different parts of *A. paniculata* are apigenin-7, 40-di-omethyl ether, carvacrol, eugenol, myristic acid, hentriacontane, tritriacontane, oroxylon A and wogonin (Rastogi and Mehrotra, 1993).

The high demand for andrographolide by the pharmaceutical industries is largely met by extraction of the compound from wild populations; however, the commercial exploitation of this compound is hampered due to its limited availability (Kanjilal *et al*., 2002). The heavy demand of andrographolide in Indian as well as international markets has motivated Indian farmers to start commercial cultivation of this medicinal plant (Kanjilal *et al*., 2002; Kataky and Handique, 2010a).

Plants use a diverse set of secondary metabolic pathways not to fulfil their primary metabolic needs in energy and biosynthetic products, but to generate a number of secondary metabolites called phytochemicals (Harborne, 1993; Gershenzon, 1994; Reymond *et al*., 2000; Hermsmeier *et al*., 2001; Kennedy and Wightman, 2011). Phytochemicals are structurally diverse

chemical compounds; based on chemical nature, they can be divided into the following major classes: (1) phenolic compounds, including flavonoids, phenolic acids, hydroxycinnamic acids, lignans, tyrosol esters, stilbenoids and alkylresorcinols; (2) terpenes, including carotenoids, monoterpenes, saponins, some modified lipid species and triterpenoids; (3) betalains, including betacyanins and betaxanthins; (4) polysulfides; (5) organosulfur compounds (Hansen and Halkier 2005; Hooper *et al.,* 2010; Menendez *et al*., 2013; Si and Liu, 2014)

Wide range of phytochemicals exhibit antioxidant and antidiabetic properties and total biological effect of plant extracts is usually attributed to the additive and synergistic effects of the complex mixture of phytochemicals present in plants (Moreira *et al*., 2017). The preclinical and clinical evaluation of these bioactive compounds may give rise to novel drugs and continuing studies of known phytochemicals may lead to improved evidence - based decisions for intensive diabetes care. Therefore, in the present work, an effort was made to confirm the presence of biologically active phytochemicals present in the various extracts *Andrographis paniculata* leaves.

Oxidative stress (formation of free radicals) is generated due to hyperglycaemic status through both enzymatic and non-enzymatic processes. These free radicals would damage cellular proteins as well as mitochondrial DNA (Brajendra *et al*., 2006). Overproduction of free radicals or reactive oxygen species (ROS) contributes to oxidative stress, that is associated with chronic degenerative diseases, including cancer, coronary artery diseases, hypertension and diabetes (Santharam *et al*., 2015 and Gafrikova *et al.,* 2014). Free radicals are formed disproportionately during diabetes due to glucose oxidation and the subsequent oxidative degradation of glycated proteins (Mehta *et al*., 2006). At present, research on correlation between antioxidant components and Type 2 Diabetes mellitus has been brought out well. Also, People reported consuming antioxidant in diet has granted to lower the growth of Type 2 Diabetes mellitus (Montonen *et al*., 2004 and Evans 2007).

Antioxidant compounds can offer a possible solution for curing serious diseases like diabetes, cardiovascular and female reproductive diseases (Gupta *et al*., 2009). Modern physicians are increasing their use of pure natural antioxidants extracted from plants to treat many important common diseases due to their proven ability to restrain specific enzymes, to stimulate a number of hormones and neurotransmitters and to scavenge free radicals (Asif, 2015). Hence, the free radical scavenging assay of *Andrographis paniculata* leaves was examined here.

Enzyme inhibitors can be a potential target in many areas of disease control and treatment, as enzymes catalyse the most important biochemical pathways. Controlled kinetics of carbohydrate digestion and monosaccharide absorption could be of great value in the avoidance of conditions such as diabetes, obesity, hyperlipoproteinaemia and hyperlipidaemia. In this aspect, amylase and glucosidase inhibitosrs are of particular importance (Alagesan *et al*., 2012). Inhibition of α-amylase, enzyme that plays a role in digestion of starch and glycogen, is considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes and obesity, as well as, dental caries and periodontal diseases (Sales *et al.,* 2012).

Fraga (2005) analyzed that the dark chocolate a rich source of flavanols could reduce blood pressure and boost the insulin sensitivity in healthy persons. One of the therapeutic approaches to treat Type 2 Diabetes is to lower the postprandial blood glucose level by inhibition of carbohydrate hydrolyzing enzyme such as alpha amylase (Gopinath *et al*., 2013). Pancreatic α-amylase enzyme plays an important role in early breakdown of complex carbohydrates into simple molecules. Modulation of α-amylase activity affects the utilization of carbohydrates as an energy source and stronger this modulation, more significant is the reduction in the breakdown of complex carbohydrates (Fatemeh, 2014).

Acarbose, voglibose and miglitol are few pharmaceutical glucosidase inhibitors currently in use that have shown considerable value in controlling hyperglycaemia. These synthetic drugs have strong inhibitory effects on both α- amylase and α-glucosidase activities; however, their several side effects have been reported, such as liver disorders, flatulence, abdominal pain, renal tumours and diarrhoea (Fujisawa *et al*., 2005 and Thinkratok *et al.,* 2014).

Managing diabetes without any side effect is still a challenge. In outlook of pharmaceutical science, though metformin and thiazolidinedione both shows better insulin resistance, they cannot be extensively used because of their unenviable aftermath. Therefore, research is been switched towards the natural resources like plants and herbs which possess antidiabetic activities with low or no side effects (Kaur *et al*., 2013). The evaluation of alpha amylase inhibitory activity is not only limited to traditional herbs or spices but also to diverse food extracts (Etxeberria *et al*., 2012). In the present study, the alpha amylase inhibition assay was carried out to detect the antidiabetic efficacy of ethanol extract of *Andrographis paniculata* leaves.

In vitro methods play an important role for the pre-clinical studies for any activity that may support *in vivo* studies (Satish *et al*., 2011). Animal models of diabetes are greatly useful and

advantageous in biomedical studies because they offer promise of new insights into human diabetes (Srinivasan and Ramarao, 2007). Identification and quantification of active constituents in the plant material may be useful for proper standardization of herbs and their formulations (Sushma *et al*., 2013). There is a need for isolation, characterization, determination of bioactivity of the lead compound for its pharmaceutical exploitation (Mariswamy *et al*., 2011)**.**

Natural products from plants are excellent sources of human pancreatic alpha amylase inhibitors. Computational molecular docking of natural products with interesting biological properties and structural diversity have often served as valuable lead drug candidates for the treatment of human diseases and also, they replace the chemically synthesized drugs which cause side effects (Maanvizhi *et al*.,2014).

Structure-based computational methods, including molecular docking, have increasingly been used in the study of biomolecular structure and function, as well as in the design of structure-based rational drugs. In particular, molecular docking contributes to the development of several inhibitors and inhibitor candidates that have been advanced to clinical trials (Kufareva and Abagyan, 2008; Torktaz *et al*., 2013; Zhang *et al*., 2014). Molecular docking is a method used to calculate the binding orientation of small molecule drug candidates to their target proteins in order to predict the affinity and activity of the small molecule. Recently docking ligands to receptors utilizing rational drug design is on the increase owing to few problems in the conventional methods of drug designing (Kitchen *et al*., 2004). In the present study, few selected diabetes proteins such as 11ß-hydroxysteroid dehydrogenase type I (11ß-HSD1; PDB ID: 1XU7), Glucagon like peptide-1 (GLP-1; PDB ID: 3IOL), Protein-tyrosine phosphatase 1B (PTP1B; PDB ID: 4Y14) were docked with natural compounds derived from *Andrographis paniculata* which offer a great expect in the identification of lead compounds for the treatment of diabetes.

11β-HSD1 (11β-hydroxysteroid dehydrogenase type I) or "cortisone reductase" which is an NADPH dependent enzyme mainly expressed in metabolic tissues such as liver, adipose tissue, and the central nervous system. In all these tissues, cortisone was reduced by HSD11B1 to cortisol that activates glucocorticoid receptors. 11βHSD1 inhibition is a tempting target for the treatment of glucortinoid-associated diseases, especially of Type 2 Diabetes mellitus (Davani, *et al*., 2004 and Andrews and Walker 1999). In the aspect of functional value of GLP-1 receptor and glycemic control maintenance it was considered to be essential of time to give an account of agonist for GLP-1 receptor which is secure than currently available substitutes in market namely Exenatide,

Liraglutide, Lixisenatide, Taspoglutide (Werner, 2014). The glucagon like peptide 1 (GLP-1) agonist is evidently employed for the regulation of Non-insulin dependent diabetes mellitus (NIDDM). At present, because of its favorable effect associated with only moderate risk of hyperglycemia in contrast to sulphonyl urea (Garber, 2011). GLP-1 hold together well with GLP-1 receptor in pancreatic b cells, and made with pro-insulin biosynthesis, insulin secretion and insulin gene transcription (Holst, 2007).

The mechanisms for the management of post-prandial rise of glucose homeostasis are done by GLP-1. Initially, GLP-1 activate cyclic AMP (cAMP) dependent pathway followed by protein kinase A (pkA) which phosphorylate the snapin protein, and that consequently make rise of calcium dependent exocytosis of insulin. pkA also induced pancreatic beta cell proliferation (Idevall-Hagren *et al*., 2010). Protein-tyrosine phosphatase 1B (PTP1B) is a pesimisstic regulator of the insulin signaling pathway and is regarded as an effective beneficial target, mainly for the treatment of Type 2 Diabetes mellitus. It has also been involved in the growth of breast cancer and has been explored as a potential therapeutic target in that avenue as well.

A huge challenge lies in developing new approaches for treating diabetes. Pancreatic alpha amylase inhibitors might be used for the design of novel functional foods with blood-glucose-lowering potential, which could be used as a complement of other antidiabetic drugs. Research work should be focused mainly on the isolation of the principal active compounds and more clinical studies are essential in order to draw concise conclusions regarding the safety and efficacy of acute and long- term administration of the extracts and their bioactive compounds in Type 2 diabetic patients (Etxeberria *et al*., 2012).

A tested pharmacological approach has been the use of synthetic compounds (drugs) such as Acarbose and Orlistat, to inhibit these digestive enzymes. However, the drugs tend to possess negative side effects ranging from diarrhoea to hepatotoxicity, which limit their use within the population (Lunagariya *et al*., 2014). Therefore, with the increasing global prevalence of obesity and Diabetes mellitus, identification of alternative enzyme inhibitors with potentially less negative side effects becomes imperative. Compounds of natural sources (such as dietary components) are more desirable as they are thought to possess lower risk of negative side effects when compared with the synthetic inhibitors (Patil *et al*., 2015).

Although there are reports regarding the *in vivo* experiment on antidiabetic activity using *Andrographis paniculata*, the *in vitro* study reports are scanty. The present research work is an

attempt to study the pharmacological effect of ethanol extract of *Andrographis paniculate* against diabetes mellitus.

Objectives:

With this background, the study was formulated with the following objectives:

- To determine the phytochemical constituents present in the *Andrographis paniculate* leaves.
- To evaluate the free radical scavenging activity of *Andrographis paniculata.*
- To analyse the *In vitro* alpha amylase inhibitory activity of ethanol extract of *Andrographis paniculata.*
- To identify the secondary active constituents, present in the *Andrographis paniculata* leaves.
- To conduct the *in silico* molecular docking studies of compounds identified against the diabetes mellitus diseased proteins.

Review of Literature

2

REVIEW OF LITERATURE

The review of literature pertaining to the study "*In vitro* antidiabetic activity and *in silico* molecular docking of Nilavembu, *Andrographis paniculata* (Burm.f) Nees" was explored and its review is presented in the following pages.

2.1. Traditional medicinal plants research- Current scenario

Medicinal plants and their derivatives have been included into traditional medicine nearly since the beginning of recorded history. But only in recent times that the extensive use of medicinal plants is beginning to gather acceptance in the more generous international domain. There are certain bottlenecks in the process, including but not narrowed to the short of quality control and toxicological studies, the necessary to increase product shelf life, and compliance with international regulatory standards that need to be rise above before their full market potential can be realized. Nature is a big source of biological and chemical varieties. The peerless and complicated structures of natural products cannot be figured out easily by chemical synthesis (Eftekhari *et al*., 2012).

Medicinal plants have been shown as a good source for development of new drugs (Gholami *et al*., 2012(a); Bahmani *et al*., 2012; Gholami *et al*., 2012(b); Ghasemi *et al*., 2012; Amirmohammadi *et al*., 2014). They have demonstrated promising effects in a wide variety of diseases such as cancer, diabetes, atherosclerosis and cardiovascular diseases, learning and cognitive complications, and wounds. Furthermore, medicinal herbs are also effective in prevention and treatment of the toxicity induced by other drugs or toxins. Medicinal plants are a rich resource of bioactive substances, antioxidants, flavonoids, and phenolic substances and have multiple health effects (Shirzad *et al.,* 2013; Shirzad *et al*., 2011; Asgary *et al*., 2014(a); Khosravi *et al*., 2012; Asgary *et al*., 2014(b); Shiao *et al*., 2008; Rafieian *et al*., 2014).

Medicinal plants provide outstanding contribution to modern therapeutics; approximately 100 plants based new drugs were introduced in the USA drug market during 1950 to 1970 including reserpine, deserpidine, vinblastine and vincristine. All these drugs are found to be derived from higher plants. From 1971 to 1990 new plant-based drugs came into being all over the world such as etoposide, eguggulsterone, artemisinin and ginkgolides. During 1991 to 1995 2%

drugs were introduced including pacitaxel, topotecan, gomishin, irinotecan etc. (Pandey *et al*., 2011). The use of herbal drugs and phytonutrients or nutraceuticals continues to expand rapidly across the world with many people now resorting to these products for treatment of various health challenges in different national healthcare settings (WHO, 2004).

Survey of literature reveals that more than 1000 companies are engaged in the production of herbal products with the annual revenues in excess of US$60 billion (Newmaster *et al*., 2013). In North America, herbal medicinal market is considered to constitute the mostly rapidly growing segment (Gutierrez *et al*., 2004), with over 29,000 herbal substances (Astin *et al*., 1998 and Kaye *et al*., 2000) generating billions of dollars in trade. These statistics are the direct indication of the rapid growth (approximately 15% per year) in the market place from natural plant products and broadening consumer base which show interest in herbal products from different countries including India. The value of botanical related trade in India is about US$10 billion per annum with the annual export of US$1.1 billion (Singh *et al*., 2003).

India is ranked third in the herbal medicine category with less than 2% global market share. The Indian market is growing at 15 - 20% per annum- Rs 7000 million or $150 million (www.indianmedicine.nic.in). Therefore, massive demand of herbal medicinal system at both national and international level has resulted in renewed interest of biologists in this field to maintain the quality and purity of herbal raw material and finished products. As the worldwide use of herbal medicinal products continues to grow and many more new products are introduced into the market, public health issues, and concerns surrounding their safety are also recognized. Although some herbal medicines have promising potential and are widely used, many of them remain untested and their use also not monitored. This makes awareness of their potential adverse effects very limited and detection of the safest and most effective therapies as well as the support of their realistic use more difficult. It has become vital, therefore, to furnish the general public including healthcare professionals with adequate knowledge to facilitate better understanding of the risks associated with the use of these products and to ensure that all drugs are safe and of suitable quality (WHO, 2002b).

Plants are the basis of both traditional medicines (TMs) and modern drug discoveries. More than 50,000 plant species are used in TM worldwide and majority of them are being used in Asian medicines. Asian medicines comprise oral-based folklore medicines (local healing system) and the scholarly TM systems (Wangchuk *et al.,* 2015). While most of the folklore medicines remain

neglected, undocumented, and are becoming rare or extinct due to fast-paced modernization, the scholarly TM systems still thrive in many Asian countries including Bhutan (Wangchuk *et al*., 2017). In the Indian system of medicine, the Vaidya is known as doctor of herbs, who makes a diagnosis of illness and compounds medicinal preparations, such as asava, aristha, churna (powders), lotions, liniments, pills, syrup, and taila. Practitioners of Ayurveda believe that every plant on the Earth has some significant medicinal property for the purpose of the good of the world; the right person just has to show you. The practitioner of Ayurveda states "Naasti Moolam Anaushadhim" has translation "Every plant on earth has a medicinal property" (Upasani *et al*., 2018) India has rich flora for the improvement of drugs from a medicinal plant (Saranraj and Sivasakthi, 2014). Perhaps, India is also gearing up, and there has been a steep rise in the global acceptance of Ayurveda, the traditional Indian medicine.

The World Health Organization has listed 21,000 medicinal plants, among which 2500 species are in India and now India is known as the largest producer of medicinal herbs (Modak *et al*., 2007 and De Luca *et al.,* 2012). Collectively, global data shows that 80% of world's population, rely primarily on ethnobotanical remedies and plant drugs, e.g., antineoplastic: camptothecin, Taxol, antimalarial: artemisinin, quinine, antigout: colchicine, analgesic: codeine, morphine, cardiac depressant: quinidine, antidiabetic: allicin, and for brain functions: caffeine, nicotine are the well-known curative agents. At the time of invention of medicinal properties of herbs, people could not think of scientific evidence, philosophical and experimental basis, molecules responsible for medicinal value and of course the currently emerging herbal genomics. Countries like India, China, Korea and Japan are now taking lead role and continuously investing in research on evidence-based traditional medicines and scientific validation of fundamental principles (Chang *et al*., 2016).

2.2. *Andrographis paniculata* (Burm.f) Nees- An overview

An herb is a plant or plant part used for its scent, flavor, or therapeutic properties, and medicinal products made from them are frequently taken to improve health as dietary supplements (Kanokwan and Nobuo, 2008). *Andrographis paniculata* (Burm. f) Nees also called as Kalmegh or "King of Bitters" belongs to the family *Acanthaceae* (Mishra *et al*., 2007) is an herbaceous plant. Mostly leaves and roots have been traditionally used over centuries for different medicinal purposes in Asia and Europe as a folklore remedy for a wide spectrum of ailments or as an herbal supplement for health promotion (Kanokwan and Nobuo, 2008).

2.2.1 Taxonomic Position

Kingdom	Plantae
Subkingdom	Tracheobionta
Class	Dicotyledonae
Subclass	Gamopetalae
Order	Gentianales
Family	Acanthaceae
Genus	*Andrographis*
Species	*paniculta*

2.2.2. Vernacular names

The vernacular names include Kirata in Sanskrit; Kriate, Kariyat, Creat in English; Alui in Bengali; Lilun kariyatun in Gujarati; Nelavemu in Telugu; Nila vembu in Tamil; Olikiryata in Marathi. They are also known in Ayurvedic, Unani and Siddha as Kalmegha, Bhunimba, Bhumi nimbak, Vishwambhar, Yavtikta, Kalpanatha, Kiryaat, Nilavembu.

2.2.3. Plant description- Morphology

It is an annual, branched, herbaceous plant erecting to a height of 30-110 cm in moist shady places with stem acutely quadrangular, much branched, easily broken, fragile texture stem. Leaves are simple, opposite, lanceolate, glabrous, 2-12cm long; 1-3cm wide with margin acute and entire or slightly undulated and upper leaves often bractiform with short petiole. Inflorescence of the plant is characterized as patent, terminal and axillary in panicle, 10-30 mm long; bract small; pedicel short. The flowers possess botanical features of calyx 5particle, small, linear; corolla tube narrow, about 6 mm long; limb longer than the tube, bilabiate; upper lip oblong, white with yellowish top; lower lip broadly cunneate, 3-lobed, white with violet markings; stamens 2, inserted in the throat and far exerted; anther basally beared. Superior ovary, 2-celled. Capsule of the plant is erect, linear-oblong, 1-2 cm long and 2-5 mm wide, compressed, longitudinally furrowed on broad faces, acute at both ends, thinly glandularhairy. Seeds are very small, sub quadrate (Medicinal plants in Vietnam. Manila, 1990; Standard of ASEAN herbal medicine, 1993; Thai herbal pharmacopoeia, 1995; Pharmacopoeia of the People's Republic of China, 1997; Mishra *et al*., 2007)

2.2.4. Ecogeographical distribution

A.paniculata or kalmegh is a tropical and sub-tropical herb native to srilanka and India. The plant flourishes best in moist shady environment but it can grow in a wide variety of habitats. Though it yields small, white and purple flowers, the spiny, dark-green stems and leaves are the primary source of medicinal value. It is presently commercially cultivated in several areas of India (Oudhia, 2009)

2.2.5. Ethnomedical importance

Ethnobotanically, the leaves and roots of *Andrographis paniculata* have been used since centuries in Asia and Europe to cure the wide spectrum of health ailments. However, the whole plant is also used for certain limited purposes. Due to its "cold property" activity, it is recommended to be used to get rid of the body heat in fevers and to dispel toxins from the body. The plants are also recommended for the use in cases of leprosy, gonorrhea, scabies, boils, skin eruptions, and chronic and seasonal fever for its high "blood purifying" properties. In addition, it is also widely used for medicinal purposes by the traditional practitioners, tribes, or community as a folklore remedies in different countries (Akbar, 2011).

2.2.6. Bioactive phytochemicals

The aerial parts of *Andrographis Paniculata* have been described for its innumerous use in the extraction of phytoconstituents; however, leaves, stems, roots, and whole plants have also been reported for phytochemicals with pharmacological activities. The compositions of phytochemicals widely differ in terms of the part used, geography, season, and time of harvesting. Sharma in 2013 reported that the highest amount of andrographolide, a major bioactive compound of *Andrographis Paniculata*, was found in the sample harvested after 110 days of cultivation followed by that just before flowering stage (130 days).

The bioactive compounds were extracted with different types of solvents such as methanol (MeOH), ethanol (EtOH), hexane, acetone, acetone-water, chloroform (CHCl3), and dichloromethane from the whole plant, leaves, aerial parts, stems, and roots. The residues were then chromatographed to get specific fractions. These fractions were further chromatographed and followed several procedures to identify specific compound. A total of 32 bioactive compounds with seven *ent*-labdane diterpenoids, twelve flavonoids, and two quinic acid derivatives have been isolated and characterized by this procedure.

Previous phytochemical studies of *Andrographis Paniculata* have reported more than 55 *ent*-labdane diterpenoids, 30 flavonoids, 8 quinic acids, 4 xanthones and 5 noriridoids, namely, andrographidoids A, B, C, D, and E (Subramanian *et al.*, 2012). Zhang *et al*. 2006 reported 3 new *ent*-labdane diterpenoids, namely, 19-norandrographolides A, B, and C from the ethanol extracts of the aerial parts of *Andrographis Paniculata*. Their structures have been established by HRESIMS and NMR spectral data in combination with X-ray crystallographic analysis; thus the 19-norandrographolide-A was identified as 3-dehydro-14- deoxy-19-norandrographolide. However, there is no report for pharmacological activity of these three compounds (Chen *et al.*, 2014).

Dua *et al.* 2004 investigated four xanthones (1,2-dihydroxy-6,8-dimethoxyxanthone; 1,8-dihydroxy-3,7 dimethoxyxanthone; 3,7,8-trimethoxy-1- hydroxyxanthone; 4,8-dihydroxy-2,7-dimethoxyxanthone) from roots of *Andrographis Paniculata* using CHCl3 fraction and purity confirmed by HPLC. Xu *et al.* 2012 isolated and established structure of 5 rare types of noriridoids with a known iridoid curvifloruside from the ethanol extracts of roots. They also assayed antibacterial activity of these compounds, but none showed any inhibitory activity (MIC > 100 μg/mL).

2.2.7. Toxicity studies

Generally, uses of *Andrographis Paniculata* as a medicine have been proved to be safe in various studies on mice, rats, and rabbits, as well as in *in vitro* assays and some clinical trials. Some conflicting results are also available. Few studies showed the toxic effect of *Andrographis Paniculata* on reproductive system by damaging the Sertoli cell in male gonads in albino rats. A dose of 25 and 50mg/kg body weight for a period of 48 days demonstrated that antispermatogenic effect (Akbarsha and Manivannan, 1993 and Kamal, 2003). However, contradictory result was also demonstrated by numerous studies [Sattayasai *et al*., 2010; Allan *et al*., 2009; Balu *et al*., 1993; Jarukamjorn *et al*., 2006; Chandrasekaran *et al*., 2009; Sharifuddin *et al*., 2012]. The safety of *Andrographis Paniculata* extracts regarding the oral acute toxicity (>17 g/Kg, LD50) (Burgos *et al*., 1997), testicular toxicity (>1 g/Kg, LD50) (Allan *et al*., 2009), and genotoxicity (5 g/Kg, LD50) (Chandrasekaran *et al*., 2009) has been reported. Due to extreme bitterness of *Andrographis Paniculata*, it may cause emesis. Some adverse effects including allergic reaction, gastric instability, fatigue, headache, loss of appetite, lymphadenopathy, diarrhea, metallic taste, and nausea are also observed in overdosing of *Andrographis Paniculata* extracts (Anju *et al*., 2012 and

Kligler *et al*., 2006). It is suggested to avoid this plant during pregnancy due to ovulation preventive effects of the plant (Zoha *et al.*,1989). To date, all trials with few exceptions were for short duration; thus, the prediction of safety for long term use would be farfetched.

2.2.8. Potential Pharmacology

Extensive use of *Andrographis paniculata* in traditional medicinal system has proven its efficacy over the past three decades. Several researches including *in vitro*, *in vivo* (animal), and clinical (human) studies have confirmed various pharmacological activities of *Andrographis paniculata* extracts and products. Andrographolide, a major *ent*-labdane diterpenoid of *Andrographis paniculata*, is the largest contributor of many pharmacological activities. Other *ent*-labdane diterpenoids (such as neoandrographolide and 14-deoxyandrographolide), flavonoids, quinic acids, and xanthones are also reported for their significant contributions (Hossain *et al*., 2014). A few of the reported works on pharmacology are summarized below.

2.2.8.1. Antimicrobial effects

Modern research has investigated the causes of extensive uses of *Andrographis paniculata* in traditional healing systems as an antimicrobial agent to treat a variety of health morbidities of infectious origin. Leelarasamee *et al.* 1990, reported that crude powder suspended in water to be devoid of *in vitro* antibacterial activity against *Salmonella*, *Shigella*, *Escherichia coli*, gram A *Streptococci*, and *Staphylococcus aureus*, even at a concentration of 25mg/mL crude powder. However, over the last 3 decades, researchers reported that different types of extracts of *A. paniculate* possess potent antibacterial activity against various pathogenic and nonpathogenic bacteria. Nakanishi *et al.* in 1965 reported antibacterial activity of aqueous methanol (50%v/v) crude extracts of whole plant against *Bacillus subtilis* and *Proteus vulgaris*. Although Nakanishi *et al.* 1965 reported the negative result against *E. coli*, ethanol extracts of aerial parts of *Andrographis paniculata* were found to be effective in inhibiting *E. coli* growth along with other ten gram positive and gram-negative bacteria species in an investigation conducted by Mishra *et al*. 2009. The aqueous extract showed significant antibacterial activity due to the combined effect of the isolated andrographolides and arabinogalactan proteins (Singha *et al*., 2003).

Researchers investigated significant antiviral activity of *Andrographis paniculata* besides other pharmacological activities in last two decades. Although they reported antiviral activity against limited viruses, such as dengue virus serotype 1 (DENV-1) (Fangkham *et al*., 2012), human papilloma virus type 16 (HPV16) and herpes simplex virus type 1 (HSV-1) (Aromdee *et al*., 2011).

Recently, Tang *et al*. 2012, reported that the methanol extract of *Andrographis paniculata* possesses significant inhibition activity against DENV-1 *in vitro* assay. Another study has revealed that andrographolide suppressed HPV16 transcription activity, leading to the reduction of E6 oncoprotein and restored p53 (Aromdee *et al*., 2011).

Several bioactive compounds such as andrographolide, neoandrographolide, dehydro andrographolide, natural derivatives of andrographolide, namely, 14-deoxy-11,12 didehydroandrographolide and 14-deoxyandrographolide, and synthetic derivatives, namely, dehydroandrographolide succinic acid monoester (DAMS), 14-´α-lipoyl andrographolide (AL-1), 3,14,19-triacetylandrographolide, and 3,9- isopropyl ideneandrographolide have been shown to have significant antiviral activity against HIV, influenza A, and HSV-1 without any significant cytotoxic effect at virucidal concentrations. Andrographolide, isolated from ethanol extracts of whole plant of *Andrographis paniculata*, showed a great promise in the treatment of HIV infections. It might be able to inhibit viral replication by interfering CDK (cyclin dependent kinase) activity, resulting in deregulation of HIV induced cell cycle (Holt and Comac 1998). The overall findings of andrographolide effects against different viruses indicate that andrographolide would be an effective agent for prevention and treatment of viral diseases.

2.2.8.2. Antiparasitic activity

Antiparasitic activity of the *Andrographis paniculata* extract is reported in certain articles. Dua *et al*. 2004, investigated both *in vitro* and *in vivo* antimalarial activity of xanthones isolated from roots of *Andrographis paniculata* against *Plasmodium falciparum* and *Plasmodium berghei*. One of the xanthones, 1,2-dihydroxy-6,8-dimethoxy-xanthone, showed substantial antiplasmodial activity during *in vitro* (4 μg/mL at IC50 value) and *in vivo* (62% parasitaemia reduction at 30mg/Kg dose) study. The water extract of dried leaves of *Andrographis paniculata* was found to be active against adult worms of *Brugia malayi in vitro* (Zaridah *et al*., 2001]. Recently, Padma *et al*. in 2011 evaluated the aqueous and methanol extracts for *in vitro* anthelmintic activity against adult earth worms *Pheretima posthuma*. The extracts showed significant results at the concentrations of 25mg/mL, 50mg/mL, and 75mg/mL. However, the clinical relevancies of the antiparasitic studies are inconclusive due to obtaining the results at high concentration that may not be feasible clinically.

2.2.8.3. Free radical scavenging activities

Reactive oxygen species (ROS) are produced during normal aerobic respiration and also in time of other metabolic actions. This can be eradicated by potent Antioxidants which eventually protect our body from diabetes, aging, and injury of kidney, liver, and cancer (Salah *et al.*, 1995). Antioxidant defense systems may only partially prevent oxidative damage (Simic, 1988). Hence, there is interest in using dietary supplements containing antioxidants to protect the components of the human body from oxidative damage. Currently, the most commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, and tert-butylhydroquinone. However, BHA and BHT have restricted use in foods because they are suspected to be carcinogenic and to cause liver damage (Sherwin *et al*., 1990). Therefore, there is growing interest in using natural additives as potential antioxidants (Jayaprakasha, *et al*., 2003). Several studies have been reported the antioxidant activities of *A. paniculata* and its constituents. Verma and Vinayak in 2008 reported that the aqueous extract of *A. paniculata* significantly increased the activities of antioxidant defense enzymes such as catalase, superoxide dismutase, and glutathione-Transferees and reduced glutathione content. The extract significantly inhibits lipid per oxidation by lowering the levels of thiobarbituric-acid-reactive substances in the liver and kidney of diabetic rats (as compared to normal rats) and also significantly increases the level of hepatic glutathione concentrations (Zhang *et al*., 2000). A pretreatment of andrographolide was reported to significantly attenuate the accumulation of thephorbol-12-myristate-13-acetate-(PMA-) induced formation of ROS and N-formyl-methionyl-leucyl-phenylalanine (fMLP-) inducing adhesion of rat neutrophils (Shen *et al*.,2000). Andrographolide exhibited free radical-scavenging ability, thus reduced oxidative stress and thiobarbituric-acid-reactive substance formation (Lin*et al*., 2009).

2.2.8.4. Anticancer activities

Andrographolide exhibited both direct and indirect effects on cancer cells by inhibiting proliferation of cancer cells, cell-cycle arrests, or cell differentiation, enhancing body's own immune system against cancer cells; and inducting apoptosis and necrosis of cancer cells (Vojdani and Erde, 2006). Dichloromethane fraction of methanol extract significantly inhibited the proliferation of HT-29 colon cancer cells. The major bioactive compound of *Andrographis paniculata*, andrographolide, isolated from dichloromethane inhibited the growth of a diverse cancer cell representing different types of human cancers (Ajaya Kumar *et al*., 2004). In contrast,

recently Aditya *et al*. in 2014 reported that methanol extract of *Andrographis paniculata* was found to be very less effective against both MCF-7 breast and HT-29 colon cancer cell lines. This low activity exhibited might be due to the low penetration power of the active principles.

Antiproliferative activities of andrographolide and isoandrographolide along with other 16 *ent*-labdane diterpenoids isolated from 85% ethanol extract of *Andrographis paniculata* against human leukaemia HL-60 cells have also been investigated by Chen *et al*. (2008). These results showed that andrographolide and isoandrographolide were more effective than others. In a recent study, Chen *et al*. (2014) identified a new flavonoid, 7, 8-dimethoxy-2-hydroxy-5-O-β-d-glucopyranosyloxyflavone, isolated from the aerial parts of *Andrographis paniculata*. This flavonoid exhibited potent antiproliferative activity against human leukaemia HL-60 cells with IC50 of 3.50 μM. Ethanol (70%) extracts and andrographolide were also found to be effective to increase the life spans of thymoma injected mice cells in an *in vivo* study (Sheeja and Kuttan, 2007). In the following year, Geethangili *et al*. (2008) showed the effective cytotoxic activity of ethanol extracts against human cancer cells including Jurkat (lymphocytic), PC-3 (prostate), HepG2 (hepatoma), and colon 205 (colonic) cancer cells.

In another study, use of andrographolide at a dose 12 μg/mL for 36 h against HL-60 cells improved 27% in G0/G1 phase cells and significantly decreased cells number at S and G2/M phase (Rajagopal *et al*., 2003). Shi *et al*. (2008) reported that andrographolide can inhibit human colorectal carcinoma (CRC) Lovo cell growth by G1–S phase arrest and induce the expression of cell-cycle inhibitory proteins p53, p21, and p16. These proteins repressed the activity of cyclin D1/Cdk4 and/or cyclin A/Cdk2, required for G1 to S phase transition.

In a recent *in vitro* study, andrographolide has been shown to suppress the growth and invasion of CRC Lovo cells and trigger apoptosis. Besides the effect of andrographolide alone, andrographolide in combination with chemotherapeutics, cisplatin, is likely to represent a potential therapeutic strategy for CRC (Lin *et al*., 2014). A novel semisynthetic analogue of andrographolide, DRF3188, exhibited anticancer activities against MCF 7 breast cancer cells at a lower dosage than andrographolide through similar mechanism (Satyanarayana *et al*., 2004). Both the compounds block cell cycle at the G0-G1 phase through induction of the cell cycle inhibitor (p27) and concomitant decrease in the levels of Cdk4. Therefore, attention has been focused on the anticancer properties of pure components of *Andrographis paniculata* and the molecular target of andrographolide that blocks G1 stage still needs to be determined.

2.2.8.5. Immunomodulatory Effect

Control of immune response by regulating nuclear factor of activated T cells (NFAT), a transcription factor essential for cytokine production during T-cell activation, is a widely known strategy. Preventing translocation of NFAT to nucleus is the target of several immunosuppressive agents (e.g., cyclosporinA, FK506) (Carretta *et al*., 2009). *Andrographis paniculata* is known to exert several immunomodulatory properties. More than two decades ago, a laboratory test demonstrated that *Andrographis paniculata* inhibited growth of human breast cancer cells similar to the drug tamoxifen (Puri *et al*., 1993). Amroyan *et al*. (1999) reported that andrographolide was effective to stop the clumping of blood platelets that lead to heart attacks and they also suggested that andrographolide has a major effect on activating the general defence functions of immune system by stimulating the production of antibodies as well as nonspecific immune responses such as increased macrophage phagocytosis.

An *in vitro* study with the increased proliferation of lymphocytes and production of interleukin-2 (IL-2) confirmed the immunostimulatory activity of *Andrographis paniculata* (Rajagopal *et al*., 2003). Three diterpene compounds of *Andrographis paniculata* isolated from dichloromethane fraction of methanol extract showed augmented proliferation and IL-2 induction in human peripheral blood lymphocytes (HPBLs) at a low concentration (Ajaya kumar *et al*., 2004). In addition, the chronic consumption of the aqueous extract of *Andrographis paniculata* also promoted the immune functions at 250mg/kg and 500mg/kg; however, 1000mg/kg dose leading to development of autoimmune reactions, anaemia and multiple myeloma (Bukoye and Musbau, 2011). *Andrographis paniculata* extract and andrographolide significantly promoted the lyses of natural killer (NK) cell-mediated target cells on day 5 after tumor induction.

Antibody dependent cell mediated cytotoxicity (ADCC) and antibody dependent complement mediated cytotoxicity (ACC) inmetastatic tumor bearing animals were also enhanced significantly compared to the control by the treatment of *Andrographis paniculata* extract and andrographolide. In addition, the levels of proinflammatory cytokines such as IL-1β, IL-6,GMCSF, and TNF-α were also effectively reduced (Sheeja and Kuttan, 2010). Carretta *et al*. (2009) demonstrated that andrographolide reduces IL-2 production, extracellular signal regulated kinase- (ERK-) 1 and ERK-5 phosphorylation induced by anti-CD3 or phorbol myristate acetate and ionomycin (PMA/Ionomycin), andNF- κB activity in Jurkat cells and this effect can be related to a reduction in NFAT activity and an increase in c-jun-Nterminal kinase (JNK) phosphorylation.

Moreover, andrographolide inhibited tumor growth in animals by stimulating the production of cytotoxic T lymphocytes (Sheeja and Kuttan, 2007). Hence, the compounds modulate the host immune systems against these cells to confer the direct cytotoxicity to cancer cells. Based on the reported immunomodulatory properties, *Andrographis paniculata* and andrographolide might be effective clinically to treat the autoimmune diseases.

2.2.8.6 Cardiovascular Effect

Cardiovascular diseases (CVDs) are the leading cause of death throughout the world. *Andrographis paniculata* is used widely for improving the cardiac health in traditional medicinal systems. Several studies have investigated its activities in cardiovascular diseases (Zhang and tan, 1996). Wang *et al*. 1997 reported that *Andrographis paniculata* is potential to increase the nitric oxide, cyclic guanosine monophosphate, and superoxide dismutase activity with declines of lipid peroxide and endothelin in an atherosclerotic rabbit model. Aqueous extracts and active constituents of *Andrographis paniculata* showed significant antihypertensive activity in both spontaneously hypertensive rats and normotensive Wister-Kyoto rats (Zhang and Tan, 1996), improved the blood pressure status in both pre- and post-experimental myocardial infarction in animals (Zhao and Fang, 1991) and exhibited platelet antiaggregation in *in vitro* (Amroyan *et al*., 1999) and *ex vivo* (Zhang *et al*., 1994) assays. The existing reports suggested that *Andrographis Paniculata* can be used as alternative source of the treatment of CVDs. Further studies are necessary to know the insight of the mechanism of actions of specific constituents of *Andrographis paniculata* and in clinical perspectives.

2.2.8.7. Antihyperglycemic Effect

Inhibitions of α-glycosidase and α-amylase activity and stimulation of insulin sensitivity are considered as effective strategies to lower the level of postprandial blood glucose. These enzymes involved in digestion and absorption of carbohydrates resulting in postprandial increase of blood glucose (Kajaria *et al*.,2013). Insulin resistance is mainly expressed by hyperinsulinemia and high blood glucose level and is associated with some metabolic hormonal abnormalities, such as dyslipidemia, abnormal uric acid metabolism, increased ovarian testosterone secretion, endothelial dysfunction, elevated procoagulant factors, and elevated inflammatory markers (Reaven, 2004).

Andrographis paniculata extracts and andrographolide effectively showed antihyperglycemic effect by (a) lowering blood glucose level through inhibition of α- glycosidase

and α-amylase (Chao and Lin, 2010); (b) increasing insulin sensitivity and thus stimulating glucose uptake and oxidation by peripheral tissues (Subramanian *et al*., 2008); (c) controlling abnormal lipid metabolism; (d) scavenging free radicals from circulation which disrupt the plasma membrane integrity resulting in decreased number of efficient plasma membrane receptors or transporter proteins necessary to uptake glucose from the blood stream (Augustine *et al*., 2014). Blood glucose lowering effect of *Andrographis paniculata* was observed in both insulin-lacking diabetic rats and normal rats in several studies (Gupta *et al*., 2008).

Andrographolide at a dose of 50mg/kg effectively decreased blood glucose level, stimulated GLUT4 translocation (Zhang *et al*., 2009), and improved diabetic rat's islet and beta cell functions (Nugroho *et al*., 2014). Glucose induced hyperglycemia (orally administered) has been prevented by water extracts of *Andrographis paniculata* in nondiabetic rats without affecting epinephrine-induced hyperglycemia (Borhanuddin *et al*., 1994). Oral administration of ethanol extracts of *Andrographis paniculata* significantly lowered the fasting blood glucose of human (Subramanian *et al*., 2008). Another bioactive compound, namely 14 deoxy-11,12-didehydroandrographolide, also showed the antihyperglycemic activity (Lee *et al*., 2010). Besides controlling blood glucose level, andrographolide also effectively prevented the onset of insulitis in a dose dependent manner and thus delayed the onset and suppressed the development of diabetes in 30-week-old NOD mice. Andrographolide also regulates the Th1/Th2/Th17 homeostasis through which it may prevent β-cell death and inhibit T-cell infiltration into pancreatic islets and thereby prevent development of type 1 diabetes (Zhang *et al.*, 2013).

Recently, Augustine *et al*. in 2014 reported that *Andrographis paniculata* decreases the blood glucose by increasing glucose utilization and oxidation, restoration of insulin signaling molecules in liver, and decreasing the serum lipid levels in high fat and sucrose induced type 2 diabetic rats without showing hypoglycemic effect. A combination of n-hexane insoluble fraction of *Andrographis paniculata* (HIFA) with curcuminoids fraction of *Curcuma xanthorrhiza rhizome* (CFC) also significantly showed the antihyperglycemic effect on high-fructosefat- fed rats (Nugroho *et al*., 2014). The combination of HIFA-CFC could be a potential source to develop an antidiabetic agent. Therefore, the identification of more antihyperglycemic compounds of *Andrographis paniculata* and combination of *Andrographis paniculata* with other medicinal plants would be a focusing point of researchers for the better treatment option of the diabetic patients.

2.2.8.8. Hepatoprotective Effect

Andrographis paniculata is widely used traditionally as a hepatoprotective agent and a stimulating agent for multiple enzymes of the liver. It is also used as an ingredient in the polyherbal preparations for the treatment of hepatic disorders in Ayurvedic and Unani medicine (Akbar, 2011). Along with different extracts of *Andrographis paniculata*, andrographolide, neoandrographolide, 14-dexoyandrographolide, and 14-deoxy-11,12-didehydroandrographolide compounds are also reported to have hepatoprotective effect (Handa and Sharma, 1990; Kapil *et al*., 1993; Roy *et al*., 2010; Akowuah *et al*., 2009). In a comparative study, the leaf extract and andrographolide was tested against the carbon tetrachloride- (CCl4-) induced hepatic microsomal lipid peroxidation. Only the leaf extract completely protected the high concentration CCl4-induced microsomal lipid peroxidation *in vitro* but not the andrographolide, which indicated that the hepatoprotective role is not solely due to the presence of andrographolide (Choudhury and Poddar, 1984). Similar effect of crude alcohol extracts of the *Andrographis paniculata* leaves against CCl4 induced liver damage was also reported by Rana and Avadhoot (1991). Handa and Sharma (1990) reported that andrographolide, methanol extract of whole plant, and andrographolide-free methanol extract improved liver histology in rats by 48.6%, 32%, and 15%, respectively, after CCl4-induced liver injury. Verma *et al.* (2003) reported the effect of ethanol extract of *Andrographis paniculata* on restoration of different enzyme after CCl4-induced liver injury. Further research using specific bioactive compounds is demanding for the better understanding of the hepatoprotective role played by the *Andrographis paniculata*.

2.2.8.9. *In silico* studies of *Andrographis paniculata*

Sharmila *et al*. 2013 Carried out the *in-Silico* Analysis of Andrographolide against two major targets of Cancer. The targets were Human Abl kinase, Human cAMP dependent protein kinase. This exhibited a minimal energy against the targets hence suggesting the stability of the compound. Comparison studies of the compound with the already available anti-cancer drugs and enzyme inhibitors, stated that andrographolide is efficient to act on the targets by exhibiting promising interactions and good scores. Enmozhi *et al*. in 2020 evaluated the compound Andrographolide from *Andrographis paniculata* as a potential inhibitor of the main protease of SARS-COV-2 (Mpro) through *in silico* studies such as molecular docking. This successfully docked against the inhibitor region of the main protease of SARS-CoV-2 virus with docking score of -3.094357 Kcal/mol, the docking score showed great binding when compared to synthetic

compounds. Correspondingly, a study was done by Siva sankari *et al*. 2016 to explore novel drug lead constituents from *Andrographis paniculata* for the treatment of Parkinson's disease. Phytoconstituents from *A. paniculata* were screened, and their activity against the monoamine oxidase B (MAO-B) protein was analyzed using Molegro Virtual Docker software. The compound neoandrographolide exhibited more potent inhibitory activity with a MolDock score of −126.78 Kcal/mol compared to that of the standard drug Zelapar which exhibited a MolDock score of −49.95 Kcal/mol. The docked pose of the compound neoandrographolide fits exactly at the active site with a maximum number of H-bond interactions.

Materials and Methods

3

MATERIALS AND METHODS

The materials used and methods adopted in the present study entitled "*In vitro* antidiabetic activity and *in silico* molecular docking of Nilavembu, *Andrographis paniculata* (Burm.f) Nees" is furnished below.

3.1. Collection of the plant

Healthy and fresh leaves of *Andrographis paniculata* was collected from Coimbatore district in Tamil Nadu, India (Fig.1).

Fig.1. *Andrographis paniculata* leaves and its powder

3.2. Preparation of leaf powder

The leaves were washed thoroughly to remove soil particles and adhering debris using sterile distilled water. Fresh leaves used for extraction were shade dried and powdered using a mechanical grinder. Fine powder was obtained by sieving (Fig.1). The powder was collected in clean air tight containers. Powdered plant material in the container was used for cold extraction.

3.3. Preparation of extract

Ten grams of leaf powder of *A. paniculata* was mixed separately with 100ml of solvents such as petroleum ether, chloroform, ethyl acetate and hydro ethanol to get the respective extracts such as PEAP (Petroleum ether Extract of *A. paniculata*), CEAP (Chloroform Extract of *A. paniculata*), EAAP (Ethyl Acetate Extract of *A. paniculata*), HEAP (Hydroethanol extract of *A.*

paniculata) the contents were periodically shaken using an electric shaker at 200rpm. After ten hours, at room temperature (37˚C), the contents were filtered through a Buchner funnel in a conical flask and it was further concentrated by evaporation by keeping the filtrate in a round bottomed flask, till the solvent completely evaporated and the extract settled down to the bottom.

3.4. Successive extraction

50gm of fresh leaves of extraction were successively extracted (cold extraction) with various solvents in the increasing order of polarity viz., petroleum ether, chloroform, ethyl acetate and hydro ethanol. The extract concentrated to a volume and solvent were allowed to dry. After drying the extracts were weighed and percentage extractive values were determined.

3.5. Qualitative phytochemical analysis

The extract was subjected to different phytochemical tests to determine the active phytochemical constituents present in the extract. Various tests to determine the presence of alkaloids, flavanoids, sterols and triterpenoids, polyphenols, glycosides, tannins, saponins, carbohydrates were performed using standard procedure (Kokate *et al*., 2007).

3.5.1. Test for alkaloids

- **Dragendroff's test:** 1ml of each test extract was treated with 1ml of Dragendroff's reagent (sodium iodide, basic bismuth carbonate, glacial acetic acid and ethyl acetate) and observed for the presence of orange brown precipitate.
- **Hager's test:** 1ml of each test extract was treated with 1ml of Hager's reagent and observed for the presence of reddish-brown precipitate.

3.5.2. Test for flavonoids

- **Shinoda test:** Few mg of each extract was treated with 1ml of ethanol and heated in boiling water bath followed by addition of 1 drop of Concentrated HCL and few pieces of magnesium fillings. The reaction mixture was incubated at room temperature for 10-15 minutes. Appearance of red color indicates the presence of flavonoids.
- **Ammonia test:** Filter paper strips were dipped in the test extracts and ammoniated change in the filter paper color to yellow indicate the presence of flavonoids. To the yellow color filter paper 10ml of H_2SO_4 was added. Disappearance of yellow color further confirmed the presence of flavonoids.

3.5.3. Test for sterols and triterpenoids

- **Libermann-burchard test:** 5ml of each test extracts were boiled with two drops of acetic anhydride and cooled, then concentrated sulphuric acid was added along the side of the test tube. Appearance of brown ring at the junction of two layers is taken as reference. If the upper layer turns green, sterols are present whereas formation of deep red color indicates the presence of triterpenoids.
- **Salkowski's test:** Each test extracts were treated with few drops of concentrated sulphuric acid and shaken well. The solution was allowed to stand for some time. Appearance of red color in the lower layer indicates the presence of sterols whereas yellow color formation in the lower layer indicates the presence of triterpenoids.

3.5.4. Test for phenols

- **Ferric chloride test:** 2ml of each test extract was treated with 2ml of 5% ferric chloride solution and formation of deep blue or black color indicate the presence of phenols.
- **Libermann's test:** 1ml of each extract was heated with a pinch of sodium nitrite. To this solution 0.5ml of dilute H_2SO_4 was added followed by addition of dilute NaOH. Formation of deep red or green or blue color indicates the presence of phenols.

3.5.5. Test for glycosides

- **Borntrager's test (Anthraquinone glycosides):** 0.5g of each extract was shaken with benzene and organic layer separated. One part of 10% ammonia solution was added to 2 parts of organic layer. A pinkish red or violet coloration in the ammonical phase indicated the presence of anthraquinone glycosides.
- **Keller Killiani test (Cardiac glycosides):** 0.5gm of each extract was added with 0.4ml of glacial acetic acid containing trace amount of ferric chloride. Contents were transferred to small test tube and 0.5ml of H_2SO_4 acid was added along the sides of the test tube. Appearance of blue color in the acetic acid layer indicates the presence of cardiac glycosides.

3.5.6. Test for tannins

- **Lead acetate test:** 5ml of each test extract was added with few drops of 10% lead acetate. Appearance of yellow color precipitate indicates the presence of tannins.

- **Ferric chloride test:** 5ml of each test extract was added 5% of ferric chloride solution. Appearance of intense green or blue color indicates the presence of tannins.

3.5.7. Test for saponins

- **Sodium bicarbonate test:** Few ml of each test extract was added with three drops of sodium bicarbonate was added and shaken well. Formation of honey comb indicates the presence of saponins.
- **Froth test:** To each test extract, 20ml of distilled water was added and agitated on a graduated cylinder for 15min. Persistence of characteristic honey comb froth at least 1cm in height for 30min indicates the presence of saponins.

3.5.8. Test for carbohydrates

- **Molish's test:** To small quantities of solvent free each test extract, few drops of 1%-naphthol in ethanol were added. Concentrated sulphuric acid was then added to the sides of the test tube. Formation of brown purple ring formed at the junction of the two liquids indicates the presence of sugars.
- **Benedict's test:** 0.5ml of each test extract was added with 2ml of Benedict's solution. Formation of reddish-brown precipitate indicates the presence of carbohydrates.

3.6. Free radical scavenging activity

The radical scavenging activities of the different extracts were measured *in vitro* against a battery of radicals namely DPPH, FRAP and H_2O_2 and reducing power assay.

3.6.1. DPPH free radical scavenging activity (Mensor *et al.*, 2001)

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. The diluted working solutions of the test extracts were prepared in methanol. About 1ml of graded concentration (20, 30, 40, 50, 75, 100 µg/ml) of extracts were taken in different test tubes and assorted with 1ml of DPPH (0.1Mm in methanol) and shaken well. This solution was then incubated in room temperature for 30 minutes. The optical density was recorded at 517 nm using UV spectrophotometer. Corresponding blank sample was prepared. Mixture of 0.5ml methanol and 0.5ml DPPH solution was used as control. The absorbance change was compared with the standard Quercetin (20-100µg/ml) and was determined. The scavenging activity was then calculated using the formula:

Radical scavenging activity (%) = Abs (control) - Abs (sample)/Abs (control) x 100, (1)

Where, Abs (control) is the absorbance of DPPH radical with methanol and Abs (sample) is the absorbance of DPPH radical with a sample extract or standard.

3.6.2. Ferric Reducing Power Assay (FRAP) (Dehghan and Khoshkam, 2012)

The FRAP (Ferric Reducing Ability of Plasma) assay was conducted according to the previously reported method of Dehghan and Khoshkam, 2012. FRAP reagent was prepared by mixing of 2.5 ml of solutions TPTZ (10 mm, (40 mm) HCL, and FeCL3 (20 mm) in 25 mm of acetate buffer (300 mm, pH 3.6), the light blue reagent contains Fe3+-TPTZ that changes to Fe2+ TPTZ as dark blue. These changes were due to the absorbance increase as monitored at a wavelength of 593 nm for different concentrations of *A.paniculata* leaf extracts in FRAP reagent. The percentage inhibition of the reaction mixture was calculated using the equation

Scavenging activity (%) = Abs (control) - Abs (sample) × 100 /Abs (control)

Where, Abs (control) is the absorbance of stannous chloride solution and Abs (sample) is the absorbance of FRAP radical with sample extract.

3.6.3. Hydrogen peroxide radical scavenging activity (Ruch *et al.*, 1989)

A solution of 45mM H2O2 was prepared in 0.1 M phosphate buffer (pH 7.4). The extracts were diluted to a concentration of 10mg in 10µl. This extract was added to 0.6ml of H2O2 solution and the final volume was made up to 3ml with phosphate buffer. After 10 minutes, the absorbance values at 230nm were recorded against a blank containing phosphate buffer without H2O2 for each sample and graded concentration (20 - 100µg/ml) of ascorbic acid served as standard solution. The percentage inhibition of the reaction mixture was calculated using the equation

Scavenging activity (%) = Abs (control) - Abs (sample) × 100 /Abs (control)

Where, Abs (control) is the absorbance of ascorbic acid solution and Abs (sample) is the absorbance of H2O2 radical with sample extract.

3.6.4. Reducing power assay (Oyaizu 1986)

Different concentrations (20, 30, 40, 50, 75, 100 µg/ml) of the extract were taken and added with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1%). These reaction mixtures were then made-up for incubation at 50 °C in water bath for 30 min, left to cool at room temperature (28 °C), and 2.5 ml of 10% TCA (Tri Chloro Acetic acid) was added to each reaction mixture. Centrifugation was done at 2000 rpm for 10 min and separated supernatant (2.5

ml) was added with distilled water. Finally, 1 ml of 0.1% ferric chloride was added and the absorbance was measured at 700nm. Ascorbic acid solution was used as standard.

3.7. Alpha-amylase inhibition assay (Nikavar, 2009)

The alpha-amylase inhibitory activity was assessed based on the colorimetric assay where Acarbose as the reference compound. Briefly, a starch solution was prepared by dissolving 0.25 g of soluble potato starch in 50 ml of deionized water (0.5% w/v). The enzyme solution made was by mixing 10 mg α-amylase in 100 ml of sodium phosphate buffer (20 mM, pH 6.9) which contains 6.7 mM sodium chloride. The extracts were subject to dissolve in DMSO to give concentrations from 100 to 500 µg/ml.96 mM 3, 5-dinitrosalicylic acid (20 ml), 5.31 M sodium potassium tartrate in 2 M sodium hydroxide (8 ml) and deionized water (12 ml) was mixed to prepare the colour reagent. The reaction was initiated by adding 500 µl of the extract to 500-µl enzyme solution and left at room temperature for 30 min. After, to 500 µl of the reaction mixture 500 µl of potato starch solution was mixed and incubated at 25 ^{0}C for 3 min. DNS reagent (500 µl) was added to it and subjected to water bath at 85 ^{0}C. After 15 min it was let to cool and was diluted with 4.5 ml of distilled water. The absorbance value at 540nm in a spectrophotometer. Finally, to prepare the blank starch solution was added after the color reagent and carried out as same above. To a control, where 500µl DMSO replacing the plant extract was added with 500µl enzyme solution and followed same procedure. Antidiabetic drug Acarbose solution at various concentrations (100 to 500 µg/ml) was a standard here. The inhibition percentage was calculated as follows:

Iα-amylase%=100× (ΔA Control–ΔA Sample) /ΔA Control

ΔA Control=A Test–A Blank

ΔA Sample=A Test–A Blank

3.8. Chromatographic analysis

Chromatographic study was done using GCMS analysis to predict the bioactive constituent which will be responsible for the Anti-diabetic and antioxidant potential of this plant. The fraction and composition of the samples were computed from the GC Peak areas. The analysis was carried out using thermo GC-Trace Ultra Ver: 5.0 GC-MS (Model Thermo MS DSQ II gas chromatograph). An Rtxi-5MS column was employed for the following conditions: Fused-DB35-MS Capillary standard Non-polar Column Dimension (30 min, ID: 0.25 mm, FILM: 0.25µm) was used. The GC temperature program was as follows: initial temperature was 75 °C, held for 2 min raised to 150 °C at a rate of 2 °C/min, then to 220 °C at a rate of 3 °C/min, and finally to 260 °C

at a rate of 6 °C/min and held for 10 min. 1:12 was the split ratio, injection temperature was 250 °C, transfer line temperature was 270 °C, and the mass spectrometer was operated at 70 eV in run time 29 min.

3.9. *In silico* studies

Computer-based methods are becoming increasingly important and complementary to wet laboratory experiments in studying the structure and function of biomolecules Jorgensen (2009) and Clark (2009). The integration of computational and experimental strategies has been of great value in the identification and development of novel promising compounds (Ferreira *et al.*, 2015). Docking studies are used at different stages of drug discovery such as to predict a ligand-receptor interaction and also to rank the compounds based on the binding energies or fitness score (Kitchen *et al.*, 2004). Molecular docking plays a significant role in structural based drug designing by predicting the binding orientation of small molecule drug candidates to their known 3D structures of the protein targets (Damayanthidevi, 2015). The compounds from the GCMS profile were proceeded with *in silico* docking assessment at selected active binding sites of the target proteins. The compounds were selected based on their prospective value and the area percentage (the suggestive of approximate quantity) as assessed by GC-MS.

3.9.1. Schrodinger suite

Schrodinger suite Maestro is Schrodinger's powerful, unified, multi-platform graphical user interface (GUI). It is designed to simplify modeling tasks, such as molecule building and data analysis, and also to facilitate the set up and submission of jobs to Schrodinger's computational programs. The main Maestro features include a project-based data management facility, a scripting language for automating large or repetitive tasks, a wide range of useful display options, a comprehensive molecular builder, and surfacing and entry plotting facilities. It contains tools for building, displaying, and manipulating chemical structures; for organizing, loading, and storing these structures and associated data; and for setting up, monitoring, and visualizing the results of calculations on these structures.

3.9.2. Retrieval of ligands

The chemical structures of the selected ligands were downloaded from pubchem database. All the ligands were prepared for binding using Lig Prep module of Maestro of the Schrödinger software suite. Lig Prep is a robust collection of tools designed to prepare high quality, all-atom 3D structures for large numbers of drug-like molecules, starting with 2D or 3D structures in SD or

Maestro format. The resulting structures can be saved in either SD or Maestro format. The simplest use of LigPrep produces a single, low-energy, 3D structure with correct chiralities for each successfully processed input structure.

3.9.3. The LigPrep Process

The LigPrep process consists of a series of steps that perform conversions, apply corrections to the structures, generate variations on the structures, eliminate unwanted structures, and optimize the structures. Many of the steps are optional and are controlled by selecting options in the LigPrep panel or by specifying command-line options. The steps are outlined below.

Converted the structure format

The input structure file present in SD format, was converted to Maestro format by sd convert. Parities specified in the SD file were converted into chiralities, which were stored as properties in the Maestro file.

Selected the structures

A subset of the input structures was selected for processing. The selection was done by mae. subset for Maestro input files and by sd convert for SD input files.

Added hydrogen atoms.

Hydrogen atoms were added in a manner that is consistent with a particular force field. This step was performed by apply h treat; the program used by the Hydrogen Treatment panel in Maestro.

Removed unwanted molecules.

Additional molecules present in the structure such as counter ions in salts and water molecules were removed by de salter. This removed all but the molecule containing the most atoms from each structure.

Neutralized charged groups.

Charged groups must be neutralized before ionization states generated. The neutralization was performed by the neutralizer, which adds or removes hydrogen ions.

3.9.4. Evaluation of drug likeliness and toxicity prediction

The ligands were scrutinized for drug-like properties by Lipinski's rule of five. This rule defines molecular properties significant for a drug's pharmacokinetics in the human body and provides the evidence concerning the exploitation of the ligands as a drug (Lipinski et al., 1997) The rules are molecular weight < 500 daltons, number of hydrogen bond donors <5 and number

of hydrogen bond acceptors < 10, calculated water partition coefficient (LogP) < 5. The ligands passing the Lipinski properties were taken for docking studies.

3.9.5. Bio activity score

The bioactivity score of the selected ligands was calculated for GPCR, ion channel, kinase, a nuclear receptor, and protease. These were done by getting SMILES notations of the selected compounds from pubchem database and fed in the online Molinspiration software version 2011.06 (www.molinspiration.com).

3.9.6. Target proteins

The Protein Data Bank (PDB) is a key resource in areas of structural biology, such as structural genomics. Most major scientific journals, and some funding agencies, such as the NIH in the USA, now require scientists to submit their structure data to the PDB. In the present study three-dimensional structure of diseased protein such as 11ß-hydroxysteroid dehydrogenase type I (11ß-HSD1; PDB ID: 1XU7), Glucagon like peptide-1 (GLP-1; PDB ID: 3IOL), Protein-tyrosine phosphatase 1B (PTP1B; PDB ID: 4Y14) were selected from the protein data bank. The receptors were equipped for docking by eliminating the inhibitors, non-catalytic waters, and all the other molecules present in the pdb files using Pymol (DeLano and Warren 2001). The proteins alone were neutralized (pH 7.4) by PROPKA. All the mislaid fragments and other errors present in the crystal structures were corrected using the Wizard Protein Preparation implemented in Maestro 11-Beta (2016) suite.

3.9.7. Molecular docking

Glide module of Schrodinger suite was used to perform docking of each selected ligand with the target protein (Friesner *et al*., 2006). The integration of position and orientation of a ligand comparative to the receptor, as well as its conformation in flexible docking, is denoted as a ligand pose. Glide ligand docking jobs required a set of previously calculated receptor grids and one or more ligand structures. Preparation of the ligands before docking is strongly recommended. If a correct Lewis structure cannot be generated for a ligand, it is skipped by the docking job. Glide also automatically skips ligands containing unparametrized elements, such as arsenic, or atom types not supported by the OPLS force fields, such as explicit lone pair atoms. Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active-site region of the receptor. The shape and properties of the receptor were represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses.

3.9.8. Examining glide data

Glide results were examined with an emphasis on visual rather than numerical appraisal. The first set of exercises used the Project Table to display the results of the SP Glide docking job, examined individual ligand poses and their contacts with the input receptor structure. The Glide Score is the one which ensures a greater docking accuracy. By default, Schrödinger's proprietary Glide Score multi-ligand scoring function is used to score the poses. The Glide Score shows a binding free energy by means of great negative values signify tighter binders. Compared with other scoring functions, Glide Score SP combines the empirical-based and force field- based scoring function to make the score more accurate.

3.10. Statistical analysis

The experiments were carried out in triplicate. The results of antioxidant and alpha amylae inhibition activities were given as mean ± standard deviation (SD).

Results

4

RESULTS

The results pertaining to the study entitled **"***In vitro* antidiabetic activity and *in silico* molecular docking of Nilavembu, *Andrographis paniculata* (Burm.f) Nees**"** are presented in the following headings.

4.1. Percentage yield of the ethanol extract

The percentage yield of the *Andrographis paniculata* leaves which is subjected to ethanol extract is shown in the Table.I.

TABLE I. Percentage yield of ethanol extract of *Andrographis paniculata* leaves

S.No.	Solvent	Empty weight of petri plate (g)	Weight of petri plate + residue (g)	Actual weight of residue	
				Extraction volume	Yield (%)
1	Hydroethanol	47.27	47.55	0.28	28.00
2	Ethyl acetate	51.34	51.56	0.22	22.00
3	Petroleum ether	54.78	54.82	0.04	4.00
4	Chloroform	47.09	47.11	0.02	2.00

Among the five solvents used, maximum percentage yield was observed hydroethanol (28%) and ethyl acetate (22%) extracts.

4.2. Qualitative phytochemical analysis

Qualitative phytochemical analysis of the leaves of *Andrographis paniculata* was carried out to identify the presence of the major phytochemicals. Results showed the presence of alkaloids, flavanoids, sterols and triterpenoids, polyphenols, glycosides, tannins, saponins, carbohydrates. Maximum intensity of the phytochemicals was observed in ethanol. Table II shows the presence or absence of various phytochemicals.

Table II. Phytochemical analysis of ethanol extract of *Andrographis paniculata* leaves

S. No.	Phytochemicals	Intensity			
		Hydro ethanol	**Ethyl acetate**	**Petroleum ether**	**Chloroform**
1	Alkaloids Dragendroff's test	+ + +	+ + +	+ + -	+ + -
	Hager's test	+ + +	+ + -	+ + -	+ + -
2	Flavonoids Shinoda test	+ + -	+ + -	+ - -	- - -
	Ammonia test	+ + -	+ + +	+ - -	- - -
3	Sterols and triterpenoids Libermann-burchard test	+ + -	+ - -	+ - -	- - -
	Salkowski's test	+ + -	+ - -	+ - -	- - -
4	Phenols Ferric chloride test	+ + +	+ + +	+ + +	+ + -
	Libermann's test	+ + +	+ + +	+ + +	+ + -
5	Glycosides Borntrager's test	+ - -	+ - -	- - -	- - -
	Keller Killiani test	+ - -	+ - -	- - -	- - -
6	Tannins Lead acetate test	+ + +	- - -	+ + +	+ - -
	Ferric chloride test	+ + +	- - -	+ + +	+ - -
7	Saponins Sodium bicarbonate test	+ + -	+ - -	- - -	- - -
	Froth test	+ + -	+ - -	- - -	- - -
8	Carbohydrate Molish's test	+ - -	+ - -	- - -	- - -
	Benedict's test	+ - -	+ - -	- - -	- - -

+ Presence of respective class compound

- Absence of respective class compound

4.3. Radical scavenging activity of *Andrographis paniculata*

The radical scavenging activities of the ethanol extract of *Andrographis paniculata* leaves were determined *in vitro* against radicals namely DPPH, FRAP and H_2O_2 and reducing power assay.

4.3.1. DPPH Assay

In the DPPH assay, the DPPH reducing activity of the ethanol extr1act of *Andrographis paniculata* was measured based on colour change which was shown due to the reduction reaction. The extent of DPPH scavenging by nanoparticle solution was significant, where the stable radical was effectively reduced to the yellow-colored compound di phenylpicryl hydrazine. The assay is based on the scavenging capacity of antioxidants towards a stable free radical α, α-diphenyl-β-picrylhydrazyl (DPPH). Among the four solvents used, ethanol extracts showed higher scavenging activity was recorded at ethanol extract compared to that of the others with the absorbance value of 0.114±0.104 (Fig.2).

4.3.2. FRAP scavenging activity

In the FRAP assay, the extent of FRAP scavenging by all the extracts was significant, where the stable radical was effectively reduced to the blue-colored compound di phenylpicryl hydrazine. Among the five solvents used, both hydro ethanol and ethyl acetate extracts showed higher scavenging activity. The least activity was shown by petroleum ether extract. The results are presented in Fig.3.

4.3.3. Hydrogen peroxide radical scavenging activity

Hydrogen peroxide, a non-radical oxidant, was scavenged more efficiently by both the hydro ethanol and ethyl acetate extracts of the leaves of *A.Paniculata* followed by the chloroform and petroleum ether extracts as given in Fig.4. Here also the petroleum ether extract exhibited least activity.

4.3.4. Reducing power ability

The presence of reductants in the ethanol extract of *Andrographis paniculata* lead to the reduction of ferric cyanide complex to ferrous form with the development of Perl's Prussian blue. This was observed at an absorbance of 700 nm. The reducing power noted was found that the ethanol extract showed highest reductive capability (Fig.5).

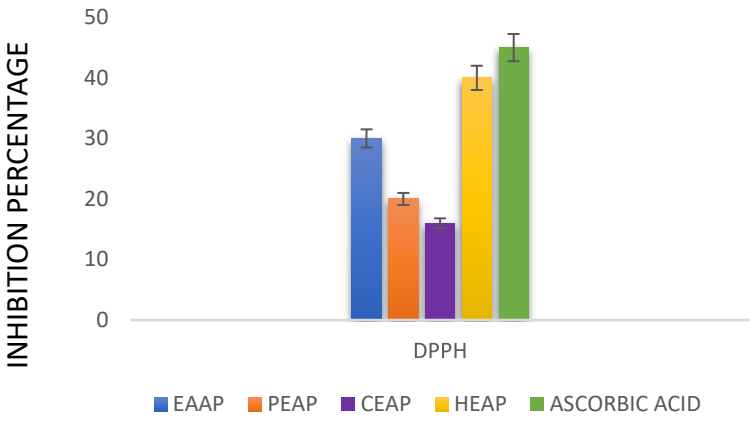


Fig.2. DPPH Assay of *Andrographis paniculata*

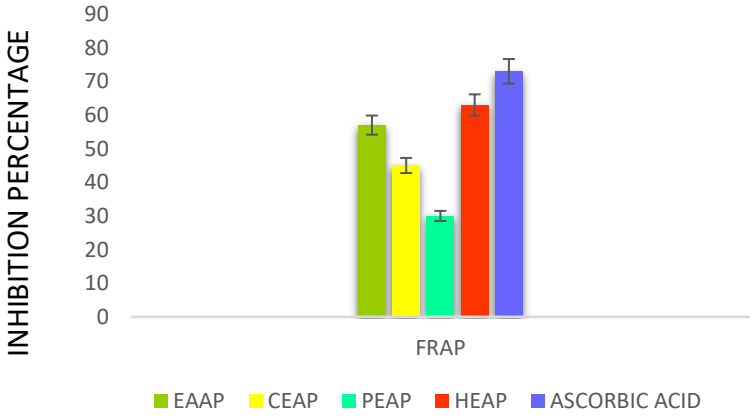


Fig.3. FRAP Assay of *Andrographis paniculata*

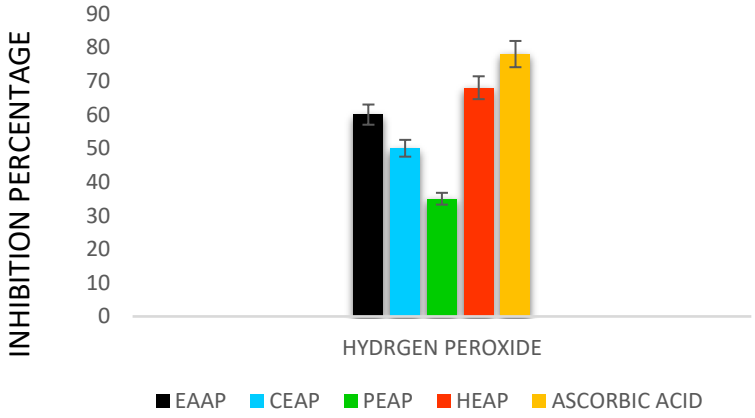


Fig.4. Hydrogen peroxide radical scavenging assay of *Andrographis paniculata*

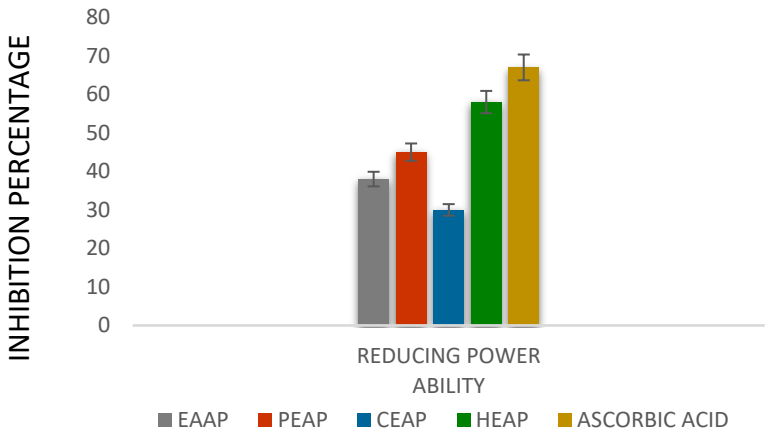
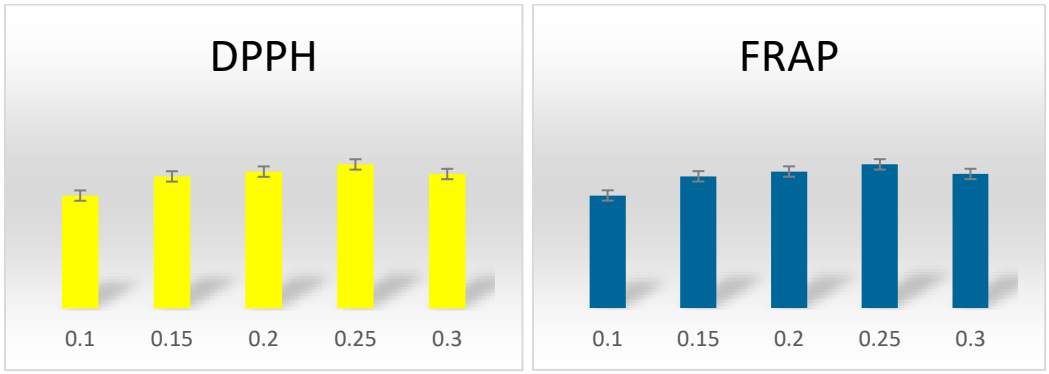


Fig.5. Reducing power ability of *Andrographis paniculata*

In the free radical scavenging assays mentioned above (DPPH, FRAP and H2O2) and reducing power assay, the four different extracts of Andrographis paniculata leaves (petroleum ether, chloroform, ethanol and ethyl acetate) were compared, among which, the ethanol extract of *Andrographis paniculata* showed the maximum activity. Therefore, further studies were carried out using the ethanol extract of *Andrographis paniculata*.

The optimum dose of the ethanol extract to be used for the subsequent phases of the study was determined. The dose was optimized using free radical scavenging assays (DPPH, H_2O_2 and FRAP) in which different concentrations (0.1mg to 0.3mg) of the ethanol extracts were compared and found that 0.25mg of ethanol extract showed the best response. Therefore, further studies were carried out with 0.25mg concentration of the rhizome. The results are shown in Fig.6.



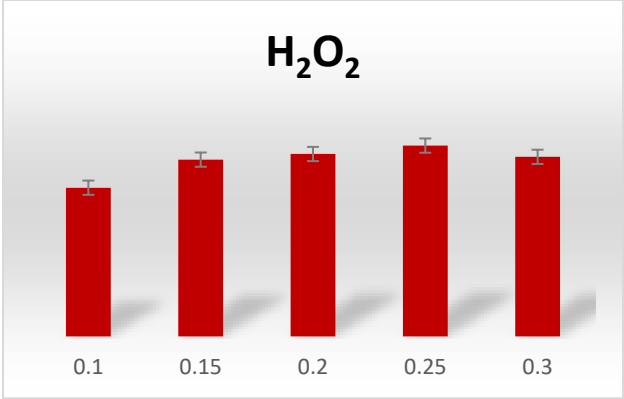


Fig.6. Free radical scavenging activity of ethanol extract of *A.paniculata*

4.4. Alpha Amylase Inhibition Assay

Ethanol extract of *Andrographis paniculata* was screened for *in vitro* amylase inhibition efficacy. Ethanol extract of *Andrographis paniculata* showed a significant α-amylase inhibition activity as shown in Table III; Fig.7. Ethanol extract of *Andrographis paniculata* and Acarbose indicated a highest inhibition percent at 500 µg/ml and lowest at 100 µg/ml revealing a dose dependent effect. The ethanol extract of *Andrographis paniculata* showed the highest absorbance of 43.21 ± 0.63 at 500 µg/ml which correlates almost to the standard acarbose which showed the highest absorbance of 46.12 ± 0.43at 500 µg/ml.

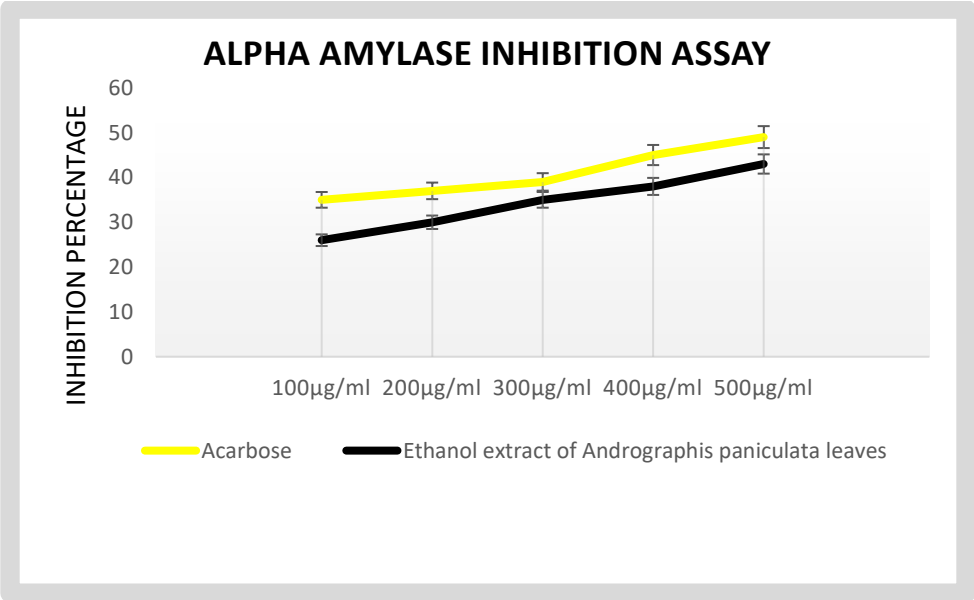


Fig.7. Alpha amylase inhibition assay of ethanol extract of *Andrographis paniculata* leaves

Table III. Inhibition % of α-amylase enzyme assay

Concentration (µg/ml)	**Inhibition (%)**	
	Acarbose	Biosynthesized AgNPs
100	30.44 ± 0.63	26.00 ± 0.16
200	33.75 ± 0.46	30.73 ± 0.39
300	36.32 ± 0.22	35.69 ± 0.13
400	41.68 ± 0.31	38.60 ± 0.41
500	46.12 ± 0.43	43.21 ± 0.63

4.5. Chromatographic analysis – GCMS

The detailed study to reveal the presence of phytocompounds were done through GC-MS analysis with help of NIST library. This study revealed the presence of major 20 compounds from 24 peaks presented in the Table IV. Some peaks were found to be broad or mixture and some were very small. The peaks of compounds and retention times present in *Andrographis paniculata* leaves identified by GC-MS analysis was reported in the chromatogram (Fig.8). Among all, the highest peak was recorded at retention time 5.857 min Di-sec-butyl Phthalate (Fig.8). The major constituents identified in the extract were Di-sec-butyl Phthalate (11.87), 2,5-Octadecadiynoic acid, methyl ester (29.39), N-Methyl-1-adamantaneacetamide (8.44), Dibromomethane (11.7) and many other compounds were identified as low level.

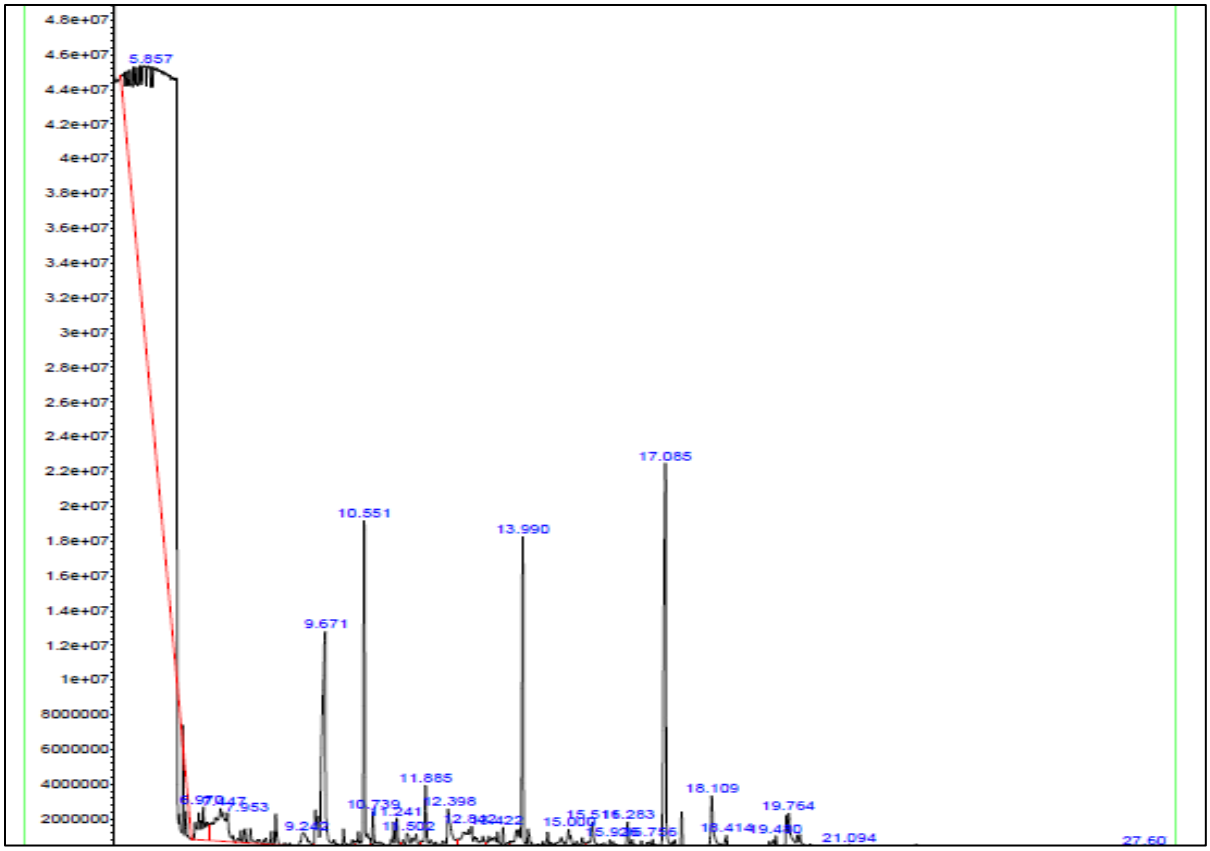


Fig.8. Mass spectra of *Andrographis paniculata* leaves

Table IV. Major bioactive compounds present in *Andrographis paniculata* leaves

S No.	Compound Name	Molecular Formula	Molecular Weight	Area %	Retention Time
1	Di-sec-butyl Phthalate	$C_7H_{17}NO$	131.22 g/mol	11.87	5.857
2	1,3,4-Thiadiazol-2-amine, 5-ethyl-	$C_4H_7N_3S$	129.19 g/mol	0.29	6.979
3	Phthalic acid, hept-4-yl isobutyl ester	$C_{10}H_{12}O_3$	180.2 g/mol	0.45	9.671
4	1-Hexyl-2-nitrocyclohexane	$C_{10}H_{20}O_2$	172.26 g/mol	3.13	10.551
5	Butanoic acid, 4-[-4-(2-methylpropyloxy) benzoylamino]-	$C_{15}H_{13}FN_2O_3$	288.27 g/mol	0.52	10.739

6	8-Methyl-3-phenyl-5-quinolinecarboxylic acid	$C_{12}H_{26}O_2$	202.33 g/mol	2.70	11.885
7	2,5-Difluoroanisole	$C_{18}H_{34}$	250.5 g/mol	0.52	12.398
8	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_{18}H_{36}O$	268.5 g/mol	0.81	13.990
9	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5 g/mol	1.17	15.516
10	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4 g/mol	5.64	15.925
11	Acetic acid, 2-propenyl ester	$C_{19}H_{34}O_2$	294.5 g/mol	0.66	17.085
12	Dodecanoic acid	$C_{18}H_{30}O_2$	292.5 g/mol	2.92	18.109
13	Phytol	$C_{12}H_{26}$	296.5 g/mol	4.16	18.414
14	2,5-Octadecadiynoic acid, methyl ester	$C_{21}H_{24}N_2O_2$	278.4 g/mol	29.39	19.410
16	N-Methyl-1-adamantaneacetamide	$C_{21}H_{24}N_2O_2$	336.4 g/mol	8.44	19.764
17	Eicosane	$C_{20}H_{42}$	282.5 g/mol	1.33	21.094
18	Pentanoic acid, 3-methyl-	$C_{12}H_{17}NO_2$	207.27 g/mol	0.79	24.573
19	9-Eicosyne	$C_{20}H_{38}$	278 g/mol	0.98	11.502
20	Dibromomethane	$C_{17}H_{24}$	184 g/mol	11.7	11.214

4.6. *In silico* molecular docking analysis

4.6.1. Selection of ligand from GC-MS profile

The resulting GC-MS from *Andrographis paniculata* had totally 20 molecules. The spectrum profile of the GC-MS data was compared with the known compounds stored in the NIST library attached to the GC-MS instrument as well as comparison of their retention indices. By

avoiding repeated structures from the sample from GC-MS results, 20 total structures were derived which were non repetitive. Twenty molecules had CAS (Chemical Abstract Services) number from Pubchem deposition and others are of ambiguities nature in the extract (Table V).

Table V. Bio-Ligands chosen for docking

S No.	**Compound Name**	**Molecular Formula**	CAS Number
1	Di-sec-butyl Phthalate	$C_7H_{17}NO$	976-78-3
2	1,3,4-Thiadiazol-2-amine, 5-ethyl-	$C_4H_7N_3S$	3999-78-8
3	Phthalic acid, hept-4-yl isobutyl ester	$C_{10}H_{12}O_3$	28564-83-2
4	1-Hexyl-2-nitrocyclohexane	$C_{10}H_{20}O_2$	7352-03-6
5	Butanoic acid, 4-[-4-(2-methylpropyloxy) benzoylamino]-	$C_{15}H_{13}FN_2O_3$	14068-53-2
6	8-Methyl-3-phenyl-5-quinolinecarboxylic acid	$C_{12}H_{26}O_2$	4925-88-6
7	2,5-Difluoroanisole	$C_{18}H_{34}$	334-48-5
8	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_{18}H_{36}O$	350039-84-8
9	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	70928-44-8
10	Pentadecanoic acid	$C_{15}H_{30}O_2$	108-84-9
11	Acetic acid, 2-propenyl ester	$C_{19}H_{34}O_2$	35189-44-7
12	Dodecanoic acid	$C_{18}H_{30}O_2$	112-39-0
13	Phytol	$C_{12}H_{26}$	1002-84-2

14	2,5-Octadecadiynoic acid, methyl ester	$C_{21}H_{24}N_2O_2$	112-63-0
16	N-Methyl-1-adamantaneacetamide	$C_{21}H_{24}N_2O_2$	301-00-8
17	Eicosane	$C_{20}H_{42}$	150-86-7
18	Pentanoic acid, 3-methyl-	$C_{12}H_{17}NO_2$	463-40-1
19	9-Eicosyne	$C_{20}H_{38}$	41191-68-8
20	Dibromomethane	$C_{17}H_{24}$	6822-38-4

4.6.2. Drug likeliness and toxicity prediction of selected molecule

Out of 20 molecules, only 10 molecules were found to satisfy drug-like properties based on Lipinski's rule of five. Lipinski's rule of five defines a molecule as drug like only if the molar weight (MW) is less than 500 Daltons (Da); the logarithm of the octanol/water partition coefficient (QPlogPo/w) is less than 5, the number of hydrogen bond acceptors (HBA) less than10 and the number of hydrogen bond donors (HBD) less than 5. The distributions of the compound MW, log P, HBA and HBD were calculated and used to assess the likely drug like nature of the compounds derived from *Andrographis paniculata* (Table VI).

Table VI showed the presence of active ligands from *Andrographis paniculata* leaves. These ligands were found to have drug like property, obeying Lipinski's rule of five. The molecular weight of the molecules was less than 500 daltons, hydrogen bond acceptors were less than 5, hydrogen bond donors were found to be less than 5 and octanol water partition coefficient less than 5.

Table VI. Compliance of compounds to computational parameters of drug likeness

No.	Compounds	MW	HBA	HBD	QPlogP3	Rule of 5 violation
1	Di-sec-butyl Phthalate	131.22 g/mol	1	0	2	0
2	1,3,4-Thiadiazol-2-amine, 5-ethyl-	129.19 g/mol	4	2	0.4	0
3	Phthalic acid, hept-4-yl isobutyl ester	180.2 g/mol	2	0	1.3	0
4	1-Hexyl-2-nitrocyclohexane	172.26 g/mol	4	1	0.9	0
5	Butanoic acid, 4-[-4-(2-methylpropyloxy) benzoylamino]-	288.27 g/mol	3	0	1.7	0
6	8-Methyl-3-phenyl-5-quinolinecarboxylic acid	202.33 g/mol	2	1	4.1	0
7	2,5-Difluoroanisole	250.5 g/mol	5	1	3.7	0
8	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	268.5 g/mol	2	1	3.6	0
9	Pentanoic acid, 3-methyl-	207.27 g/mol	4	1	2.9	0
10	N-Methyl-1-adamantaneacetamide	336.4 g/mol	2	0	4.4	0

4.6.3. Bioactivity score

The bioactivity score of nineteen ligands which was chosen from *Andrographis paniculata* leaves was calculated for GPCR (G-Protein Coupled Receptor) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitors (Table VII). These properties serve as an indication of excellent pharmacological activities *in vivo.*

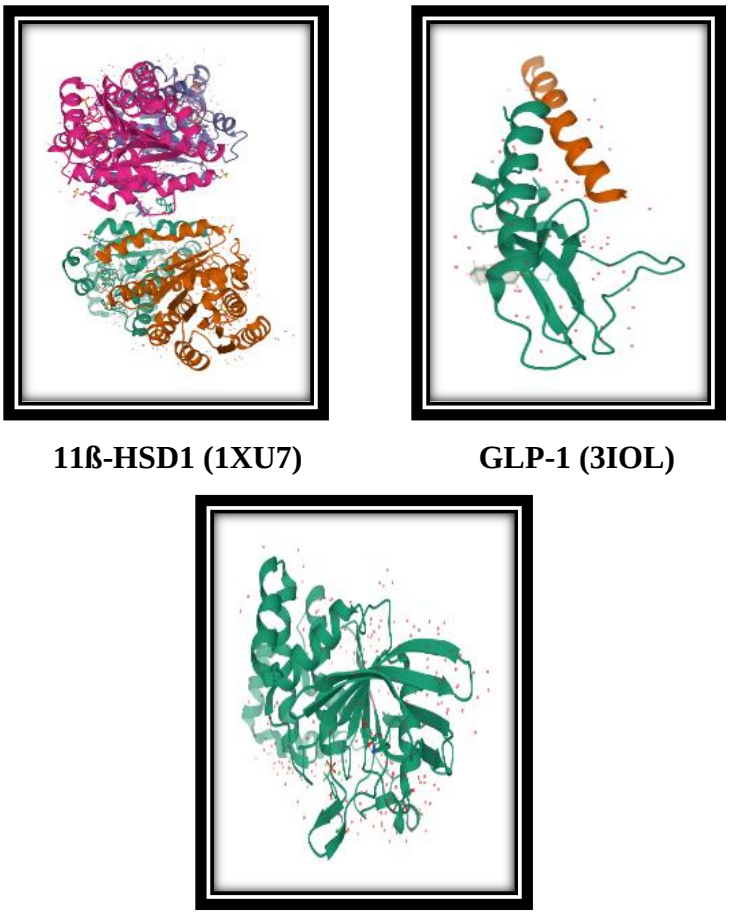
Table VII. Bio activity score of chosen ligands

No.	Compounds	GPCR Receptor	Ion Channel Receptor	Protein Kinase Receptor	Nuclear Receptor	Protease Receptor	Enzyme Inhibitor
1	Di-sec-butyl Phthalate	-2.10	-1.37	-2.28	-2.41	-2.47	-1.62
2	1,3,4-Thiadiazol-2-amine, 5-ethyl-	-1.59	-0.96	-2.25	-1.60	-1.53	-0.65
3	Phthalic acid, hept-4-yl isobutyl ester	-2.06	-1.89	-2.29	-2.63	-2.23	-2.19
4	1-Hexyl-2-nitrocyclohexane	-3.74	-3.77	-3.49	-3.81	-3.66	-3.52
5	Butanoic acid, 4-[-4-(2-methylpropyloxy) benzoylamino]-	-1.08	-0.63	-0.91	-0.80	-1.33	-0.66
6	8-Methyl-3-phenyl-5-quinolinecarboxylic acid	-0.46	-0.14	-1.03	-0.45	-0.56	-0.07
7	2,5-Difluoroanisole	-0.22	-0.15	-0.25	-0.20	-0.38	-0.15
8	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	-0.40	-0.15	-0.67	-0.50	-0.39	-0.11
9	Pentanoic acid, 3-methyl-	0.33	0.09	-0.22	0.23	0.27	0.15
10	N-Methyl-1-adamantaneacetamide	-0.54	-0.12	-0.82	-0.56	-0.67	-0.28

4.6.4. Diseased Protein for diabetes

The selection of target protein for diabetes was made through literature survey. There are three protein retrieved from PDB. Accordingly, the protein structures were retrieved on basis of many criteria such as single monomer, less than 2 resolution, Homo sapiens and single domain in

the protein complex. All ligands chosen from *Andrographis paniculata* were docked against the three chosen diabetes proteins such as 11ß-hydroxysteroid dehydrogenase type I (11ß-HSD1; PDB ID: 1XU7), Glucagon like peptide-1 (GLP-1; PDB ID: 3IOL), Protein-tyrosine phosphatase 1B (PTP1B; PDB ID: 4Y14). The structure of the diabetes proteins derived from PDB is shown in Fig.9.



11ß-HSD1 (1XU7) **GLP-1 (3IOL)**

PTP1B (4Y14)

Fig.9. 3D structure of proteins prepared using Schrodinger suite

4.6.5. Docking of target proteins with ligands

To investigate the detailed intermolecular interactions between the ligand and the target protein, an automated docking program maestro 9.6 was used. It performs grid-based ligand docking with energetics and searches for favorable interactions between one or more typically small ligand molecules and a typically larger receptor molecule, usually a protein. Three-dimensional structural information on the target proteins was taken from the Protein Data Bank (PDB). Protein preparation was done to delete water molecules not associated with active sites and

to regenerate the native status and also for the addition of hydrogen atoms. The compounds extracted from *Andrographis paniculata* obtained from GC-MS analysis were docked into the active site of the proteins. A correlation was calculated by Glide score.

The diseased proteins, 11ß-HSD1, GLP-1and PTP1B were selected from protein data bank. Protein was interacted with screened ligands from *Andrographis paniculata* leaves. The entire docked complex was visualized using XP visualizer. The hydrogen bonding interactions between the ligands and the proteins were also visualized.

The results of the docking studies showed that the ligands Butanoic acid, 4-[-4-(2 methyl propyloxy) benzoylamino]-, 8-Methyl-3-phenyl-5-quinolinecarboxylic acid, and N-Methyl-1-adamantaneacetamide- exhibited gliding interaction with 11ß-HSD1 with the gide score of -6.904, -6.122, -5.944. 1,3,4-Thiadiazol-2-amine, 5-ethyl-, 2,5-Difluoroanisole, 8-Methyl-3-phenyl-5-quinolinecarboxylic acid exhibited gliding interaction with GLP-1 with the glide score of -4.03, -3.701, -3.664. Also, 4H-Pyran-4-one, 2,3-dihydro-3,5 dihydroxy-6-methyl-, Pentanoic acid, 3-methyl-, 8-Methyl-3-phenyl-5-quinolinecarboxylic acid exhibited gliding interaction with PTP1B with the glide score of -8.318, -5.725, -5.569 (Table VIII).

Table VIII. Summary of glide score of the top ranked poses in each protein

Protein	Screened Ligands	Glide Score
11ß-HSD1	Butanoic acid, 4-[-4-(2-methylpropyloxy) benzoylamino]-	-6.904
	8-Methyl-3-phenyl-5-quinolinecarboxylic acid	-6.122
	N-Methyl-1-adamantaneacetamide-	-5.944
GLP-1	1,3,4-Thiadiazol-2-amine, 5-ethyl-	-4.03
	2,5-Difluoroanisole	-3.701
	8-Methyl-3-phenyl-5-quinolinecarboxylic acid	-3.664
PTP1B	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	-8.318
	Pentanoic acid, 3-methyl-	-5.725
	8-Methyl-3-phenyl-5-quinolinecarboxylic acid	-5.569

The inference of the score indicated that 8-Methyl-3-phenyl-5-quinolinecarboxylic acid is the unique molecule from *Andrographis paniculata* which can act as diabetes drug for the analyzed diabetes proteins. For the calculation of the results mainly three parameters were considered which

include G-score, H-bond energy, and residual interaction. Based on these parameters the binding affinity of ligand towards the binding protein was determined. The more negative value of G-score indicated good binding affinity of the ligand with protein. More hydrogen bonds in the structure indicated that the ligand was having good binding mode to the protein. Residual interaction showed where the ligand exactly binds to amino acid of the protein. The G score, hydrogen bond and residue interaction of the 6-ligand protein complex is described in Tables VIII-X and Fig. 10-12.

4.6.5.1. Docking interaction between 11ß-HSD1 and Butanoic acid, 4-[-4-(2-methylpropyloxy) benzoylamino]-

The docking profile of 11ß-HSD1 to Butanoic acid, 4-[-4-(2-methylpropyloxy) benzoylamino]-from *A.paniculata* showed five oxy- hydroxy group interaction at ASP 92, LYS 125, HIS 93 and ARG130of 11ß-HSD1. The Glide score was -6.904 and number of hydrogen bonds was found to be 5. A Glide score of -6.904 and 5 hydrogen bonds proves the efficacy of Butanoic acid, 4-[-4-(2-methylpropyloxy) benzoylamino]- in docking 11ß-HSD1 (Table IX and Fig.10).

Table IX: Docking interaction between 11ß-HSD1 and Butanoic acid, 4-[-4-(2methylpropyloxy) benzoylamino]-

Protein complex	Amino Acid	Protein Atom	Ligand Atom	G Score	No of Hydrogen Bonds
11ß-HSD1- Butanoic acid, 4-[-4-(2-methylpropyloxy) benzoylamino]-	ASP 92 LYS 125 ARG130 HIS 93	H	O	-6.904	5

4.6.5.2. Docking interaction between GLP-1 and 1,3,4-Thiadiazol-2-amine, 5-ethyl-

The docking profile of GLP-1 to 1,3,4-Thiadiazol-2-amine, 5-ethyl- from *Andrographis paniculata* showed six oxy- hydroxy group interaction at LYS125, ALA126, ASP 92, GLN 171, HIS 93 and ARG 130 of GLP-1. The Glide score was -4.03 and number of hydrogen bonds was found to be 6. A Glide score of -4.03 and 6 hydrogen bonds proves the efficacy of 1,3,4-Thiadiazol-2-amine, 5-ethyl- in docking GLP-1 (Table X and Fig.11).

Table X. Docking interaction between GLP-1 and 1,3,4-Thiadiazol-2-amine, 5-ethyl-

Protein complex	Amino Acid	Protein Atom	Ligand Atom	G Score	No of Hydrogen Bonds
GLP-1 - 1,3,4-Thiadiazol-2-amine, 5-ethyl-	LYS125 ALA126 ASP 92 GLN 171	H	O	-4.03	6

4.6.5.3. Docking interaction between PTB1B and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-

The docking profile of PTB1B to 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- from *Andrographis paniculata* showed two oxy- hydroxy group interaction at MET 793 and GLN 791 of PTB1B. The Glide score was -8.318 and number of hydrogen bonds was found to be 2. A Glide score of -8.318 and 2 hydrogen bonds proves the efficacy of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- in docking PTB1B (Table XI and Fig.12).

Table XI. Docking interaction between PTB1B and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-

Protein complex	Amino Acid	Protein Atom	Ligand Atom	G Score	No of Hydrogen Bonds
PTB1B - 4H Pyran-4-one, 2,3-dihydro 3,5 dihydroxy-6 methyl-	MET 793 GLN 791	H	O	-8.318	2

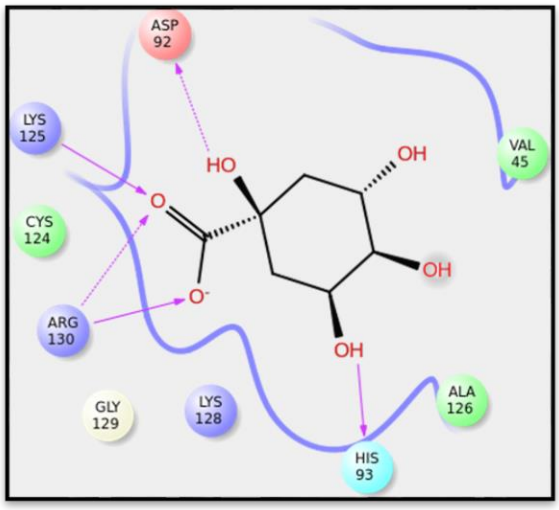


Fig.10. 3D plot of complexed 11ß-HSD1 and Butanoic acid, 4-[-4-(2-methylpropyloxy) benzoylamino]-

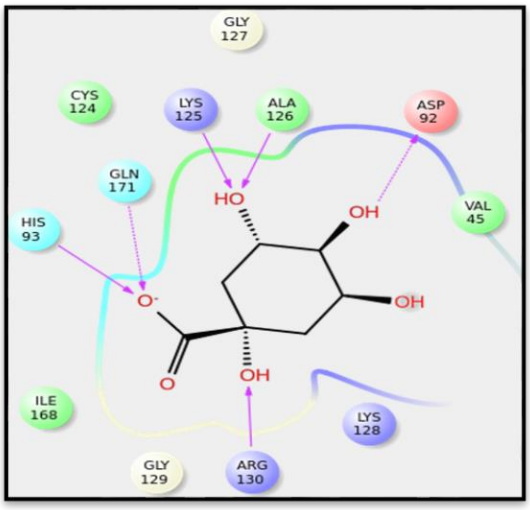


Fig.11. 3D plot of complexed GLP-1 and 1,3,4-Thiadiazol-2-amine,5-ethyl-

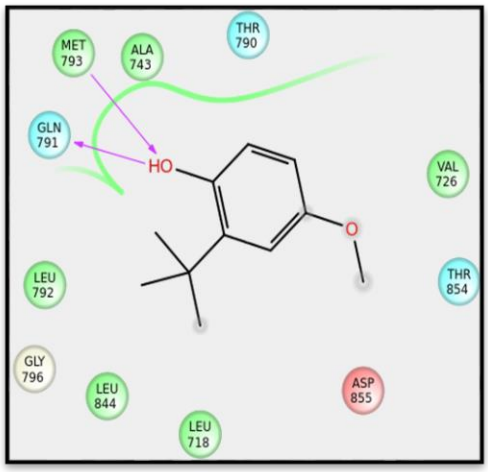


Fig.12. 3D plot of complexed PTB1B and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-

The results revealed that 8-Methyl-3-phenyl-5-quinolinecarboxylic acid was the one marked to be docked with all the 3 proteins 11ß-HSD1, GLP-1, PTP1B with the gliding score of -6.122, -3.664, -5.569. In the three formed complexes by 8-Methyl-3-phenyl-5-quinolinecarboxylic acid with three different proteins 8-Methyl-3-phenyl-5-quinolinecarboxylic acid-11ß-HSD1complex exhibited the highest glide score of -6.122 with 5 hydrogen bonds at residues such as LYS 125, ASP 92, ARG 130 and HIS 93 (Fig.12). 11ß-HSD1 was the one associated well with the incidence of diabetes.

Discussion

5

DISCUSSION

From ancient times, plants have been used as an important source of medicine due to the presence of a wide spectrum of biologically active compounds. The popularity of the herbal drugs is increasing worldwide generally and particularly in the developed countries but one of the obstacles in its acceptability is lack of standard quality control profile. World Health Organization (WHO) emphasized including the physicochemical and the phytochemical evaluation of crude drug materials for developing standardized quality control profile of herbal medicine (Singh and Ali, 2012).

The most important things for consumers about medications are purity, safety, potency, and efficacy. Thereby, standardization and quality control of herbal medicines and raw material are always required. The popularity of the herbal drugs is increasing worldwide generally and particularly in the developed countries but one of the obstacles in its acceptability is lack of standard quality control profile. World Health Organization (WHO) emphasized including the physicochemical and the phytochemical evaluation of crude drug materials for developing standardized quality control profile of herbal medicine (Alam and Saqib, 2015).

Andrographis paniculata (Burm.f) Nees., which belongs to the family Acanthaceae, is an important traditional medicinal and dye yielding plant. It is an important ethno- medicinal plant native to southeastern Asia, i.e., India, Sri Lanka, Pakistan, Java, Malaysia and Indonesia but it is cultivated extensively in India, China and Thailand, the East and West Indies, and Mauritius (Kanokwan and Nobuo, 2008). Different parts of the plant showed a wide spectrum of biological activities. Writings on use of *Andrographis paniculata* plant in natural systems of medicine, Ayurveda, Siddha, Unani, Tibbi, and Amchi have tempted us to take up and study about this plant.

The usage of herbal extracts or active compounds in food, cosmetic and pharmaceutical industries have being increased in the last years, so that the biological and phytochemical study of medicinal plants is essential and an interesting area of research. Phytochemical evaluation is efficient way to predict the plant drugs and discover various components present in different polar solvents. Extractive value helps in indication of chemical constituents and in estimation of specific

constituents soluble in particular solvents in a crude drug. Among the five solvents used in this study, maximum extraction yield was observed in both ethanol (28%) and ethyl acetate (22%) extract of the leaves. Recent report supports this observation, extraction of *Terminalia catappa* Linn. leaves with ethanol and ethyl acetate gave highest yield such as 5.7% and 0.28% respectively (Tercas *et al.*, 2017). The differences in the extract yields can be attributed to variable solubility of phytoconstituents based on their varied chemical composition in the plant (Khan *et al.*, 2015).

Phytochemicals, the plant-derived bioactive compounds form the basis of many modern-day drugs. Many conventional drugs that are widely used in mainstream medicine are derived from phytochemicals. Preliminary phytochemical screening is helpful in the prediction of nature of drugs and also useful for the detection of different constituents present in solvents of different polarity. An extraction procedure with superlative efficiency with respect to time/yield ratio is fundamental to accurately quantify the phytoconstituents (Celeghini *et al.*, 2001).

The qualitative phytochemical study of *Andrographis paniculata* was carried out in different solvents revealed the presence of alkaloids, flavonoids, phenols, carbohydrates, sterols, tannins and tri terpenoids. In the present study the maximum intensity of phytochemicals was reported in ethanol extract followed by ethyl acetate extract. The results are in agreement with previous studies. Several phytochemical studies on *Andrographis paniculata* leaves and stem extracts in different solvents have conducted. Dulara *et al.*, (2019) revealed the presence of saponins, flavonoids, alkaloids, phenols, glycosides, sterols, tannins and tri terpenoids in the methanol extract as well as petroleum ether extract of *Andrographis paniculata* leaves. Ethanolic extract of *Ipomoea batatas* (L.) Lam., reported to possess high number of alkaloids, flavonoids, phenols, sterols, tannins, anthraquinones and coumarins followed by ethyl acetate extract (Pochapski *et al.*, 2011).

The various phytochemical compounds detected are known to have beneficial importance in medicinal science. The flavonoids are reported to possess anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities (Oomah, 2003). Alkaloids have been used as ant malarial, pain killer and to manage heart diseases. Glycosides serve as defense mechanism against predation by many microbes (De *et al.*, 1999). Steroids are known for their cardiotonic activities, insecticidal and antimicrobial properties (Callow, 1936). Phenols and tannins have antioxidant properties and saponins were used in hypercholesterolemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory and weight loss. Apart from this *A. paniculata* is found to contain the major

components like andrographolide, neoandrographolide and andrographanin are reported to have medicinal property (De-Lucca *et al*., 2005)

Free radicals are molecules with one or more unpaired electron in their outermost orbit. These highly reactive molecules attack the nearest stable molecule to obtain an electron. Subsequently, the targeted molecule becomes a free radical itself and initiates a cascade of events that can ultimately lead to cellular damage (Agarwal *et al*., 2008). This free radical toxicity has been implicated in the biomolecular damage of the proteins, lipids and DNA. In this scenario, the determination of free radical scavenging is important. Free radical-induced oxidative damage is involved in the pathogenesis of many chronic and degenerative diseases, such as cardiovascular disease, cancer, diabetes, neurodegenerative disease and ageing.

Reactive oxygen species (ROS), including superoxide free radical, hydrogen peroxide, hydroxyl free radical and singlet oxygen, play a key role in the oxidative damage of the diseases, which may result in DNA mutations, protein inactivation, lipid peroxidation, cell apoptosis or abnormal proliferation, eliciting the occurrence of diseases from the cellular and molecular levels (Rahman *et al*., 2011). Antioxidants are substances capable of scavenging ROS and protecting from oxidative damage. Antioxidant compounds like phenolic compounds, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.

The nutrient antioxidant deficiency is one of the causes of numerous chronic and degenerative pathologies and cancer. Each nutrient is unique in terms of its structure and antioxidant function (Willcox *et al.,* 2004). Naturally occurring phytochemical antioxidants have occupied a prominent position as effective antioxidants for the prevention and/or treatment of several disorders and diseases among humans (Hollman *et al.*, 2011; Spatafora and Tringali, 2012). The premise for this has been the antioxidant actions of the phytochemicals as free-radical scavengers, oxidative stress relievers, and lipoperoxidation inhibitors (Scalbert *et al.*, 2005).

DPPH is a relatively stable free radical scavenger which converts the unpaired electrons to paired ones by hydrogen proton donation. DPPH (α, α-diphenyl-β- Picrylhydrazyl;C18H12N5O6, M=394.33) is a commercially available organic nitrogen radical. Plant extracts contain compounds capable of donating protons to the free radicals leading to inhibition at a higher concentration in all the solvent extracts. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine (Contreras-Guzman and Strong,

1982). In the present study, DPPH shows a strong absorption maximum at 517nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stochiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517nm.

Our results suggest that different extracts have different activities and maximum activity was found in ethanol and ethyl acetate extract followed by petroleum ether and chloroform extracts. The more antioxidant present in the extract, the more DPPH reduction will occur. Scavenging of DPPH radical in this study indicates the potency of the plant extracts in donating hydrogen proton to the lone pair electron of the radicals. The method has proven the effectiveness of the extracts in a concentration-dependent manner. The antioxidant activity observed in the DPPH radical scavenging assay may be as a result of the phytoconstituents present in the plant extracts.

A study has shown that the andrographolide exhibited significant antioxidative property (IC_{50}: 3.2 µg/mL) by its ability to scavenge a stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as compared to known antioxidants such as ascorbic acid, butylated hydroxy toluene (BHT), and the plant extract (Krithika *et al*.,2013). Begum *et al.* in 2017 screened 15 medicinal plants including *Andrographis paniculata* for antioxidant activity using DPPH assay and measured its absorbance at 490 nm. The results revealed that the three extracts–hexane, ethanol and methanol of the plant *Andrographis paniculata* have best scavenging activity with minimum scavenging activity of 24% and maximum was 96%. Singh *et al.,* (2015) support this observation by demonstrating the DPPH scavenging potential of ethanolic extract of *Indigofera tinctoria* Linn. Furthermore, another study supports this observation as the ethanolic extract of *Lepisanthes rubiginosa* L. leaves showed pronounced scavenging activity with IC_{50} value 31.62µg/ml (Hasan *et al.,* 2017). Similarly reports have evidenced that hydroalcoholic extract of *Psidium cattleianum* Sabine leaves exhibited free radical scavenging activity with IC_{50} value (15.9 µg/mL) obtained by the DPPH method (Alvarenga *et al.,* 2013). Furthermore, Balakrishnan *et al.,* (2011) reported that ethanolic extract of *Muntingia calabura* leaves exhibited significant DPPH scavenging activity in a dose dependant manner with the IC_{50} value of 8.5 µg.

The ability of plant extract to reduce ferric ions was determined in FRAP assay. The change in absorbance at 593 nm owing to the formation of blue colored Fe^{+2} - tripyridyltriiazine (TPTZ)

compound from the colourless oxidized Fe^{+3} form by the action of electron donating antioxidants. The FRAP values of ethanol and ethyl actate extracts are significant comparable to ascorbic acid. Thus, it can be reported that ethanol and ethyl actate extracts may act as free radical scavenger, capable of transforming reactive free radical species into stable non radical products.

Our findings are supported by previous reports. The ethanol extract (527.79 ± 16.26 umol) of *Cynara scolymus* leaves showed highest reducing ferric capacity followed by ethyl acetate extract (508.29 ± 5.24 umol) in FRAP assay (Salem *et al.*, 2017). Another study by Karthivashan *et al.,* (2013) revealed that ethanolic extract of *Moringa oleifera* leaves possess highest FRAP and molybdenum scavenging activities. Ethanolic extracts of *Allium sativum* L. leaves showed best antioxidant activities in FRAP and DPPH assays compared to bulb and flower extracts (Nencini *et al.,* 2011). Biapa *et al.,* (2007) revealed that ethanolic and hydrolysed extracts of *Amphimas pterocaroides, Harungana madagascariensis, Myrianthus arboreus,* and *Cussonia barteri* had highest scavenging activities in FRAP and DPPH assays.

H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, it is a weak oxidizing agent, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. It can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. In our study, the decomposition of H_2O_2 by *A.paniculata* leaves was significant in ethanol and ethyl acetate extracts with followed by chloroform and petroleum ether extracts.

Our results are in consistent with previous reports. Ethanolic extract of *Indigofera tinctoria* Linn. reported to possess highest scavenging activities in case of DPPH, metal chelation and hydroxyl radical-scavenging assays (829, 659 and 26.7 µg/mL) (Singh *et al.,* 2015). A study by Pracheta *et al.* (2011) revealed highest H_2O_2 scavenging activity ethanolic extract of *Euphorbia neriifolia* leaves comparable to BHT and ascorbic acid standards.

The reducing power is generally associated with the presence of reductones which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Shimada *et al*., 1992). Plant extracts has the reducing ability to transform Fe_{3+} to Fe_{2+} and reductones are responsible for it. During the reducing power assay, the presence of reductants (antioxidants) in the tested samples would result in reducing Fe_{3+}/ferricyanide complex to the ferrous form (Fe_{2+}). The amount of Fe_{2+} can be monitored by measuring the formation of perl's

prussian blue at 700nm. In the present investigation, the reducing power of the various extracts suggest that, ethanol extract of the *Andrographis paniculata* showed highest reducing ability and this activity may contribute significantly towards the total antioxidant effect of the ethanol extract of the sample.

This is due to existence of phytochemicals in the extract (Lin *et al.,* 1996). Pham *et al.,* 2019 studied the antioxidant property of the *C. roseus* root extract and its two sub-fractions including saponin-enriched (SE) and aqueous (AQ) fractions using reducing power and reported that the saponin-enriched fraction had the highest antioxidant capacity. The Antioxidant power of the saponin enriched fraction was 1.6–2.2 times and 5.2–6.9 times greater than those of the root extract or the aqueous fraction, respectively. These findings can be explained by the high levels of saponins and phenolics in the saponin-enriched fraction in comparison with those in the root extract or the aqueous fraction, and these compounds were found to have strong correlation with antioxidant power. Phenolic compounds like flavonoids protect human body from harmful free radicals, whose formation is associated with the normal natural metabolism in aerobic cells (Amic *et al*., 2003). It was reported that polyphenols have been found to protect mammalian and bacterial cells from cytotoxicity induced by hydrogen peroxide (Nakayama *et al*., 1993). Consequently, Suparna *et al*. 2014 described the phytochemical screening of *Andrographis paniculata* extracts prepared in methanol, DCM and n-hexane using HPTLC which showed the presence of flavonoids in higher amounts besides other phytochemicals where these phytochemicals were responsible for good radical scavenging.

Earlier reports support our observation. Moukette *et al.,* (2015) reported that ethanolic extract of *Monodora myristica* barks had highest $DPPH^{+}$, OH^{+}, NO^{+}, $ABTS^{+}$ scavenging activities comparable with BHT and vitamin C antioxidants. Similarly, ethanol extract of *Vernonina patula* showed highest reducing power ability (1.928 at 100 µg/mL) when compared to (2.449) standard ascorbic acid (Hira *et al.,* 2013). Morrison and Twumasi (2010) revealed that the selected leafy vegetables showed strong antioxidant properties with respect to their free radical scavenging activity and Fe^{3+} reduction ability with hydro-ethanol extracts indicating higher antioxidant potential compared with their respective methanol extracts. In the present study, the data obtained from radical scavenging activity have established that the ethanolic extracts of *Andrographis paniculata* exhibited outstanding scavenging effects on DPPH, H_2O_2 and FRAP radicals.

The Diabetes Mellitus has become a growing problem in the contemporary world. Diabetes mellitus is a metabolic disorder with increased blood glucose level. Since T2DM is associated with neurological and cardiovascular problems which results from metabolic disorders arising from hyperglycemia, the most precarious remedy is to maintain the blood sugar level within the normal range. Several epidemiological and clinical studies indicate a direct relationship between hyperglycemia and long term microvascular and macrovascular complications, which develop as the disease progresses, gradually decrease quality of life of diabetic patients (Mahesh *et al.*, 2016). This may eventually lead to multiple organ damage and syndromes (Rahimi *et al.*, 2005).

The most important digestive enzyme is pancreatic alpha-amylase, a calcium metalloenzyme that catalyzes the hydrolysis of the alpha-1, 4 glycosidic linkages of starch, amylose, amylopectin, glycogen and various maltodextrins and is responsible of most of starch digestion in humans producing glucose as a final product (Tundis *et al*., 2010). Alpha-amylase catalyzes in the assimilation of dietary starch, releasing oligosaccharides which are then further broken down to glucose, promptly absorbed by the body. It is a linear chain consisting of repetitive glucose units linked by α-1, 4-glycosidic linkage while branching occurs every 15-45 glucose units where α-1, 6 glycosidic bonds are present. α-Amylase has become an enzyme of crucial importance due to its starch hydrolysis activity for the production of glucose and fructose syrup from starch. α-Amylase catalyses the first step in this process. Therefore, the inhibition of this enzyme is one of the effective strategies in diabetes therapy (Sundarram and Murthy, 2014). Inhibitors of saccharide hydrolyzing enzymes (α-amylase and α glucosidase) have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with Type 2 Diabetes Mellitus (Gin and Rigalleau, 2000).

In the present study, inhibition of alpha amylase by ethanol extract of *Andrographis paniculata* leaves were studied and showed a highest inhibition percent at 500 µg/ml and lowest at 100 µg/ml revealing a dose dependent effect. Our results corroborate, with the findings of Subramanian *et al.* 2008 who have conducted the alpha-glucosidase and alpha-amylase inhibition with the ethanolic extract of *Andrographis paniculata* and andrographolide. The extract showed appreciable alpha-glucosidase and alpha-amylase inhibitory effect in a concentration-dependent manner in the ethanol extract than andrographolide. Begum *et al.* in 2017 screened the ethanol extract of *Andrographis paniculata* for Inhibition of alpha glucosidase assay. The percentage inhibition showed a dose dependent increase at 1–3 mg concentrations of *Andrographis paniculata*

extract. The percentage inhibition varied from 60%-31% for highest and lowest concentrations respectively. Thus, by the inhibition of alpha glucosidase enzyme, the extract of *Andrographis paniculata* containing numerous natural bioactive compounds play a great role in reducing the rate of digestion of carbohydrates which then decrease the levels of blood glucose and thus, diabetic conditions are maintained. Similarly, Deepak in 2020 evaluated the in vitro antidiabetic activity of the Hexane, DCM and Methanol extracts of *Andrographis paniculata* Nees. The extracts were analyzed for antidiabetic potential using a systemic study platform which includes in vitro assays namely alpha glucosidase inhibitory activity and alpha amylase inhibitory activity. Here, examining the IC_{50} values of all the analytes, methanol extract was found to be a strong inhibitor of enzymes with IC_{50} being 27.97 mg/ml and 55.1 mg/ml for the α-glucosidase and α-amylase respectively. This could be defensible that the presence of some constituents (alkaloids, phenols, saponins, and terpenoids) in the extract were accountable for being effective inhibitors of α-amylase (Kim, 2010).

Many bioactive compounds from different plants have been reported to have hypoglycemic effect, in that mostly phenolics and triterpenoids such as oleanane, ursane, lupane, and flavonoids have a positive correlation as antidiabetic agents (Tundis *et al.,* 2010; Sales *et al*., 2012; Brahmachari., 2011). The presence of triterpenoids and phenolics in the ethanol extract of *Andrographis paniculata* leaves might have attributed to the highest enzyme inhibition activity. Hence, the triterpenoids of this plant may be responsible for enzyme inhibitory activity. Apart from that polyphenolic compounds may interact or inhibit specific positions in enzymes thereby reducing the potency of α-amylase and α-glucosidase (Rohn *et al.,* 2002). The presence of flavonoid compounds may act against diabetes mellitus either through their capacity to avoid glucose absorption or to improve glucose tolerance by competitive inhibition of sodium-dependent glucose transporter-1(Shimizu, 2000). Another possible mechanism followed by flavonoid compounds (luteolin, kaempferol, chrysin, and galangin) to control blood glucose levels is the inhibition of α-amylase and α-glucosidase activity in the intestine (Kim., 2000).

Due to above reasons, the ethanol extract of *Andrographis paniculata* leaves showed comparable results with that of acarbose. With the help of results in correlation with previous reports it can be hypothesized that the significant enzyme inhibitory activity of ethanol extract of *Andrographis paniculata* leaves interferes or delay the absorption of dietary carbohydrates as well as disaccharides in the small intestine, leading to the suppression of meal-induced increase of

plasma glucose. Hence, it may useful in the management of T2D. Based on the lead fractions obtained from *in-vitro* studies, *in-vivo* study for further confirmation of the obtained results is recommended.

Though there are the factors that phytoconstituents play a lead role in the performing biological activities the comprehensive study was done to examine the bioactive compounds which are responsible for all these pharmacological activities. GC-MS analysis have been applied by many researchers to identify the possible bioactive components present in the plant extracts and herbal preparations, which might be useful for the identification of lead compounds for the development of new pharmaceutical drugs.

In the present study, leaves of *Andrographis paniculata* showed twenty-four major peaks comprising twenty significant compounds by GC-MS analysis. The major constituents identified in the extract were Di-sec-butyl Phthalate (11.87), 2,5-Octadecadiynoic acid, methyl ester (29.39), N-Methyl-1-adamantaneacetamide (8.44), Dibromomethane (11.7) and many other compounds were identified as low level. The biotic activities of major 20 compounds have been described in earlier times. Some peaks were found to be broad or mixture and some were very small. The results are in partial agreement with earlier reports of the same plant. Dulara *et al*. in 2019 presented a GCMS report with 5 extracts namely, Methanolic Leaf Extract, Methanolic Stem Extract, Methanolic Callus Extract, Petroleum Ether Leaf Extract and Petroleum Ether Stem Extract. Some of the phytochemicals detected here are eicosane, phytol, Di-sec-butyl Phthalate, Phthalic acid, hex-3-yl isobutyl ester and Tetradecanoic acid, 10,13-dimethyl-, methyl ester. These compounds were said to possess antimicrobial activity, antidiabetic, antioxidant and anticancer activity.

Likewise, Somarathinam *et al.,* in 2018 reported a data on GCMS analysis of ethanol extract of *A. paniculata* with core chemical compounds such as Ethanol, 2-(9, 12-octadecadienyloxy)-, (Z, Z)-, Hexadecanoic acid, ethyl ester, Octadecenoic acid, methyl ester , Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol), dasycarpidan-1-methanol, acetate (ester) and Cyclohexane, 1,1'-dodecylidenebis[4-methyl-]. These types of secondary metabolites would act as hypoglycemic agents which helps in reducing blood sugar level in an adequate range. Phytol is found to have antimicrobial, antioxidative and antidiabetic property (Kumar *et al.,* 2010). 5-Eicosene have also proven to show antioxidant and antidiabetic effects (Hamidi *et al.,* 2012). Similarly, the phenol showed a significant antioxidant effect (Lee *et al*., 2007).

Due to time and cost limitations of experimental approaches, a number of predictive approaches attempt to predict target-ligand relationships *in silico*. For *in silico* analysis, ligand optimization was carried out. Molecular docking was done to examine the drug efficiency potential of the compounds. Molecular docking is the computational method for structure-based drug designing which gives an idea about the proper and stable conformation of ligand and target protein and also states about suitable protein ligand interactions. Molecular docking provides a comprehensive insight into molecular mechanisms of biological processes. Influence of molecular docking is highly experienced in the field of structure-based drug discovery, wherein docking is vital in validating novel lead compounds.

Hence, to get an insight about the mode of interaction between the binding site of proteins and the active compounds identified from *Andrographis paniculata,* docking studies were conducted. From the bioactive molecules obtained from GC-MS analysis, 10 unique ligands were retrieved. They were found to obey the obligatory limits by satisfying the lipinski's rule of five and thereby recommended to be orally active. The molecular docking studies and Lipinski's rules facilitate drug development avoiding expensive post clinical experiments (Lalitha and Shubashini 2011). The bioactivity scores also attributed for drug potentiality. From the selected compounds which are supposed to be docked with four different proteins the best scoring against the target proteins was mean to be with better agonistic effect leading to Antidiabetic potency. Low docking energy indicates high binding ability. 8-Methyl-3-phenyl-5-quinolinecarboxylic acid was the potent inhibitor in comparison to all others where the docking score stands top with all the selected proteins.

The therapeutic applications of amino acids have noted to play a vital role in metabolic disorders, renal failure, cardiology, neurological disorders and congenital defects (Sensi *et al.*, 1989). The hydrogen bond interaction from the docking study indicated the involvement of amino acid such as ASP 92, LYS 125, ARG130, HIS 93, LYS125, ALA126, ASP 92, GLN 171, HIS 93, ARG 130, MET 793, GLN 791etc. As said above Compounds with less ligand-receptor binding energy and high interactions with the receptor (<6 Å bond lengths) were predicted to be most effective. Compounds exhibiting interaction with insulin receptor may be capable in tempering hyperglycemia by insulin signaling (Mehta *et al.,* 2016). These molecules can be considered to prove as beneficial through their direct action on insulin receptor.

Our results are supported by the earlier findings. Rahman *et al.,* (2020) performed investigations into the binding activities of andrographolide, curcumin, catechin, gallic acid, and rosmarinic acid with human glucokinase protein (PDB ID: 1V4S) using a molecular docking approach with Autodock Vina. The binding affinity was highest for andrographolide (12.1 kcal mol), followed by catechin (10.2 kcal mol), rosmarinic acid (8.6 kcal mol), curcumin (7.8 kcal mol), and gallic acid (5.6 kcal mol). It is anticipated that andrgapholide as least energy molecule which fulfils the Lipinski rules of five, hence it could inhibit the human glucokinase protein and improve insulin action for recovery of Type-2 Diabetes (Tolambiya and Mathur 2017). An *in-silico* study on antidiabetic activity of bioactive compounds in *Euphorbia thymifolia* Linn. were reported against the proteins such as 11-β HSD1, GFAT, PTP1B, SIRT6. This showed the binding capacity of all three family bioactive compounds: tannin, flavonoid and terpenoid in *E. thymifolia* on 4 proteins related to Type 2 DM in humans. Thereby, tannin and flavonoid families included first seven compounds. Those compounds are β-amyrine, taraxerol, 1-*O*-galloyl-β-d-glucose, corilagin, cosmosiin, quercetin-3-galactoside and quercitrin which were selected for pharmacophore analysis. This also proves that the 11β HSD1 was the best receptor for binding of tannin and flavonoid family (Vo *et al*., 2016).

The present study is in line with Ogunwa (2016) who studied molecular interaction and inhibitory potential of plant-derived phenolic compounds scirpusin B, cassigarol E, epicatechin gallate and sarcoviolin on human α-amylase and compared with standard drugs used for the treatment of Type 2 Diabetes. Computational ligand docking revealed that these compounds possessed higher binding affinity (-9.2Kcal/mol, -9.0Kcal/mol, -8.9Kcal/mol and -8.3Kcal/mol respectively) and showed higher inhibitory potentials on human α-amylase as compared with reference compounds (acarbose and miglitol) having -7.5Kcal/mol and -5.1Kcal/mol binding energy respectively. Consequently, Begum *et al.* in 2017 screened about 15 medicinal plants including *Andrographis paniculata* for *in silico* docking study of diabetes with the target protein Peroxisome proliferator–activated receptors (PPARγ) and revealed that andrographolide and Deoxy 11,12-didehydro andrographolide showed docking score of -5.488 and -4.109.

Our experimental studies showed that ethanol extract of *Andrographis paniculata* leaves are harboring various bioactive compounds such as terpenoids, phenols and flavonoids which have protective effect against free radicals. Docking studies revealed that *Andrographis paniculata* possess drug candidates against diabetes targets. All these findings support the strong

chemotherapeutic efficacy of *Andrographis paniculata* leaves which has been used as traditional drug preparations for various disorders.

Summary and Conclusion

6

SUMMARY AND CONCLUSION

Traditional medicine has a long history of serving peoples all over the world. Natural products play an important role in the field of new drugs research and development. Recent studies have also revealed promising results from using of plants in the treatment or prevention of a wide variety of hard curable diseases such, athrosclerosis, diabetes, cardiovascular diseases, neurological disorders and cancer. Medicinal plants with antioxidant activity have been shown to counteract these situations and always been considered as a healthy source of health promotion.

Andrographis paniculata is one of the most vital and infrequent ancient books buried ethnomedicinal plant, traditionally used in Siddha and Ayurveda systems. It is a flowering shrub-like species distributed widely in the tropical regions and used as folk medicine by tribes. The plant parts are used in the treatment of gastropathy, gastralgia, stomach ulcer, wounds, gout, inflammation, hernia, sarcocele, fever, diabetes and cancer. The present research work is focused on the free radical scavenging and antidiabetic studies an using *Andrographis paniculata* leaves extracts and exploration of its pharmacological applications.

The summarized results of the present research work are given below:

- In the present study, preliminary screening and qualitative phytochemical analysis of different solvents (petroleum ether, chloroform, ethanol, ethyl acetate) revealed the presence of nine major phytoconstituents namely alkaloids, flavanoids, sterols and triterpenoids, polyphenols, glycosides, tannins, saponins, carbohydrates
- Nutrient antioxidants have been shown to be involved in detoxification of the reactive oxygen species (ROS) and play an important role in helping endogenous antioxidants for the neutralization of oxidative stress.
- Naturally occurring phytochemical antioxidants have occupied a prominent position as effective antioxidants for the prevention and/or treatment of several disorders and diseases among humans.
- The premise for this has been the antioxidant actions of the phytochemicals as free-radical scavengers, oxidative stress relievers, and lipoperoxidation inhibitors.

- In the present study, free radical scavenging assays established that ethanol and ethyl acetate extracts of *A.paniculata* leaves exhibited stupendous scavenging effects on DPPH, FRAP, H_2O_2 radicals and reducing power ability compared to other solvent extracts.
- Therefore for further study, the ethanol extract of *A.paniculata* leaves were carried out.
- Diabetes is a metabolic disorder where human body does not produce sufficient insulin or properly use it, a hormone that is required to convert sugar, starch and other food into energy.
- The increasing prevalence of Type 2 Diabetes Mellitus and the side effects observed with the commercially available antidiabetic drugs requires investigation of new therapeutic approaches for controlling postprandial glucose levels.
- The use of carbohydrate digestive enzyme inhibitors from natural resources could be a possible strategy to block dietary carbohydrate absorption with less adverse effects than synthetic drugs in the treatment of diabetes.
- Hence, in the present study the antidiabetic potential of alpha amylase inhibitors in the ethanol extract of *Andrographis paniculata* leaves were examined.
- The results of antidiabetic study revealed that ethanol extract of *Andrographis paniculata* showed 93 and 95 % α-amylase inhibition activity respectively at 400 and 500 µg/ml compared to the standard.
- It is observed that increasing the concentration of the extracts increased the inhibition of α-amylase enzyme and revealed the antidiabetic potential of α-amylase assay has been suggested as a facile screening tool for establishing the antidiabetic nature of commonly used medicinal plants and to provide scientific evidence for their traditional ethnobotanical knowledge.
- Though a variety of techniques can be used to determine and estimate the presence in medicinal plants of bioactive substances that provide definite physiological action on the human body, Chromatography undoubtedly, is the most useful and popular tools used for this purpose.
- GC-MS analysis revealed the presence of 20 significant compounds including hydroxyl and carbonyl groups. This valuable information of the plant can be used as noteworthy parameters to develop phytotherapeutic drugs and to ensure standardization for its safety and efficacy.

- The present study aimed to find the drug targets for diabetes from the plant compounds of *Andrographis paniculata* leaves. Ligand optimisation from the GCMS profile of revealed 10 compounds that were subsequently taken in for docking studies with highly influential diabetes proteins such as 11ß-HSD1, GLP-1 and PTP1B.
- Out of various complexes docked using Schrodinger glide module, 3 complexes (11ß-HSD1 interacted with Butanoic acid, 4-[-4-(2-methylpropyloxy) benzoylamino]-; GLP-1 interacted with 1,3,4-Thiadiazol-2-amine, 5-ethyl; PTB1B interacted with 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-) showed highest glide score, H-bond energy and residual interaction.
- Based on this observation, four unique compounds (Butanoic acid, 4-[-4-(2-methylpropyloxy) benzoylamino]-, 8-Methyl-3-phenyl-5-quinolinecarboxylic acid, 1,3,4-Thiadiazol-2-amine, 5-ethyl and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-) were suggested as good inhibitors for the analyzed diabetes proteins.
- Altogether this study throws light on the possibility to recommend *Andrographis paniculata* as a potential therapeutic agent owing to its remarkable radical scavenging and antidiabetic properties. According to the *in vitro* and *in silico* studies *Andrographis paniculata* leaves stands out as a promising orally bioavailable anticancer drug candidate for diabetes. Further *in vivo* studies on *Andrographis paniculata* plant are needed for successful discovery of safe and efficient drug.

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