K.Kalaiarasi is currently working as Assistant Professor in department of biotechnology, Sree Narayana Guru college, Coimbatore. She has got 8 years of teaching experience in both UG & PG classes. Her research area is molecular biology, r-DNA technology, Pharmacology.

Plants have many phytochemicals and secondary metabolites which Posses various bioactivities like antimicrobial, antioxidant, anti-diabetic properties. In this study, the aim was to characterize and compare the Phytochemical, Antioxidant and Antidiabetic Activities. The phytochemical screening of plant extracts were performed, and the Comparison was done between two solvents Ethanol and Chloroform where ethanol shows more active compounds. The Antioxidant activity test is performed by DPPH ASSAY, FRAP TEST, NITRIC OXIDE ASSAY, HYDROGENPEROXIDE ASSAY. The Antidiabetic activity test is performed by ALPHA AMYLASE ACTIVITY TEST.

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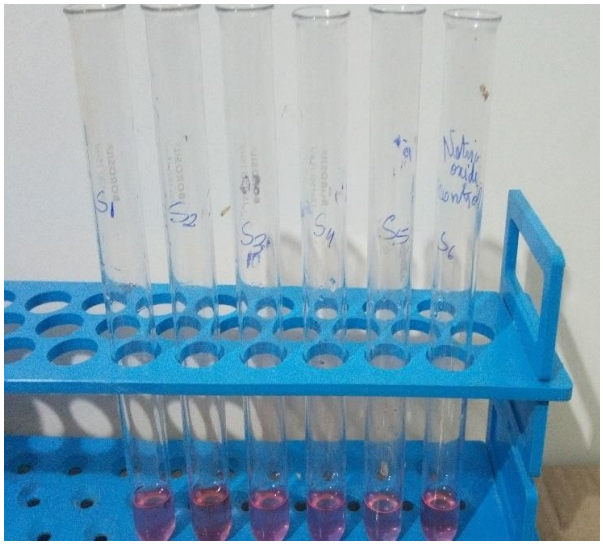

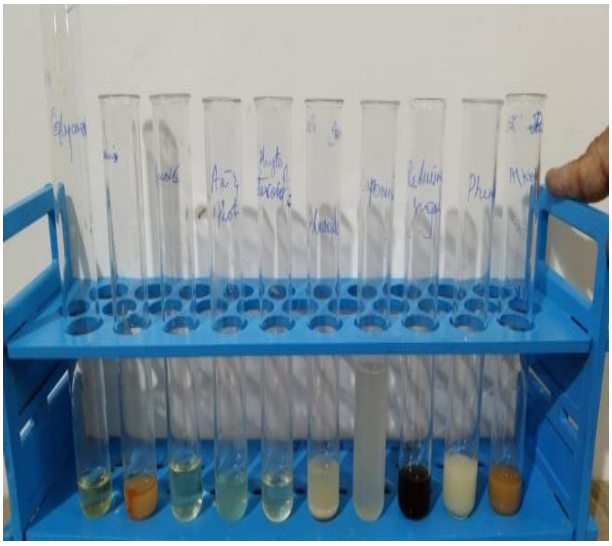

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* Acknowledgement from the third author (Keerthy K)

Dedicated

To

My* Beloved Parents,

Family

&

Friends

* Dedication from the third author (Keerthy K)

Table of contents

Table of figures

Table of tables

List of abbreviations

%	: Percentage
°C	: Centigrade
A. paniculata	: *Andrographis paniculata*
BCBT	: Beta Carotene Bleaching Test
DMSO	: Dimethyl Sulfoxide
DPPH	: 1,1-diphenyl-2- picrylhydrazyl
FRAY	: Ferric Reducing Antioxidant Power
HS-GC	: Head Space Gas Chromatography
IDF	: International Diabetics Federation
NMR	: Nuclear Magnetic Resonance
PE	: Plant Extract
TEAC	: Trolox Equivalent Antioxidant Capacity
WHO	: World Health Organization

Evaluation of phytochemical characterisation, antioxidant and antidiabetic activities of *Andrographis paniculata*

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Abstract

Plants have many phytochemicals and secondary metabolites which Posses various bioactivities like antimicrobial, antioxidant, anti-diabetic properties. In this study, the aim was to characterize and compare the Phytochemical, Antioxidant and Antidiabetic Activities. The phytochemical screening of plant extracts were performed and the Comparison was done between two solvents Ethanol and Chloroform where ethanol shows more active compounds. The Antioxidant activity test is performed by DPPH ASSAY, FRAP TEST, NITRIC OXIDE ASSAY, HYDROGENPEROXIDE ASSAY. The Antidiabetic activity test is performed by ALPHA AMYLASE ACTIVITY TEST.

Keywords: Plant extracts; Antioxidant; *Andrographis paniculate*; Antidiabetic.

1. Introduction

Plants have been an important source of medicine. Traditional medicinal plants have therapeutic value worldwide due to its affordability, accessibility, and cultural importance. The World Health Organization (WHO) estimate up to 80 % of people depending mainly on traditional remedies such as herbs and spices for their medicine. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups.

Medicinal plants are considered as a rich resource of ingredients which can be used in drug development either phermacopoeial or synthetic drugs. A part from that these plants play a critical development of human cultures around the world wide. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values.

In many developing countries, it is estimated that about two third of the population relies heavily on traditional practitioners and medicinal plants to meet primary healthcare needs. As a result of the numerous problems associated with orthodox drugs, many plant species are now been revalued by researchers based on variation in plant species and their therapeutic chemical principles.

Andrographis paniculata Wall (family Acanthaceae) is one of the most popular medicinal plants used traditionally for the treatment of array of diseases such as cancer, diabetes, high blood pressure, ulcer, leprosy, bronchitis, skin diseases, flatulence, colic, influenza, dysentery, dyspepsia and malaria for centuries in Asia, America and Africa continents. It possesses several photochemical constituents with unique and interesting biological properties.

In Ayurvedic medicinal system, tribals of Tamil Nadu, India use this herb for a variety of ailments like dysmenorrhoea, leucorrhoea, pre-natal and post-natal care, complicated diseases such malaria, jaundice, gonorrhoea and general ailments like wounds, cuts, boils and skin diseases.

1.1 *Andrographis paniculata*

Andrographis paniculata (family Acanthaceae) is one of the most popular medicinal plants used traditionally for the treatment of array of diseases such as cancer, diabetes, high blood pressure, ulcer, leprosy, bronchitis, skin diseases, flatulence, colic, influenza, dysentery, dyspepsia and malaria for centuries in Asia, America and Africa continents. Andrographis might work by stimulating the immune system. It might

also prevent influenza viruses from binding to cells in the body. In recent times, commercial preparations of this plant extracts are also used in certain countries. However, the preparations yet need to be standardized for their better efficacy. The aerial part of AP is most commonly used; its extracts contain diterpenoids, diterpene glycosides, lactones, flavonoids, and flavonoid glycosides. Whole plant leaves and roots are also used as a folklore remedy for different diseases in Asia and Europe. The plant grows as an erect herb to a height of 30– 110 cm (12–43 in) in moist, shady places. The slender stem is dark green, square in cross-section with longitudinal furrows and wings along the angles. The lance- shaped leaves have hairless blades measuring up to 8 cm (3.1 in) long by 2.5 cm (0.98 in). The small flowers are pink, solitary, arranged in lax spreading racemes or panicles. The fruit is a capsule around 2 cm (0.79 in) long and a few millimeters wide. It contains many yellow-brown seeds. The seeds are subquadrate, rugose and glabrous. The flowering time is September to December.

Medicinal uses

The whole plant has been used for several applications such as anti-dote for snake-bite and poisonous stings of some insects, and to treat dyspepsia, influenza, dysentery, malaria and respiratory infections. The leaf extract is a traditional remedy for the treatment of infectious disease, fever-causing diseases, colic pain, loss of appetite, irregular stools and diarrhea. In Malaysia, a decoction of the aerial parts is used to treat common cold, hypertension, diabetes, cancer, malaria and snakebite. In Ayurvedic medicinal system, tribals of Tamil Nadu, India use this herb for a variety of ailments like dysmenorrhoea, leucorrhoea, pre-natal and post-natal care, complicated diseases such malaria, jaundice, gonorrhoea and general ailments like wounds, cuts, boils and skin diseases. ‘

1.2 Phytochemical analysis

The word ‘Phytochemical’ derived from Greek which means plant chemicals. Mainly the phytochemical testing is used for extraction, screening and identification of medically useful compounds in the given sample because the phytochemicals are promoted for the prevention and treatment of many health conditions, such as cancer, heart disease, diabetes and high blood pressure. The phytochemicals help to prevent the formation of carcinogens or act on cells that suppress cancer development. Some bioactive substances derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds. The phytochemicals are primary and

secondary compounds. Protein, common sugars and chlorophylls are primary and alkaloids, tannin, phenolic compounds are secondary compounds. Terpenoids contain pharmacological activities like anti– inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti- viral and anti-bacterial activities. Alkaloids are an aesthetic agent. The antioxidant and secondary metabolites found in plant body such as fruits. The secondary metabolites influence the antioxidant activity.

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defence mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds. The plant showed potential therapeutic action in curing liver disorders, common cough and colds in human. The presence of important phytochemicals in *A. paniculata* make the plant useful for treating different ailments and have a potential of providing useful drugs of human use. The quantitative determination of pharmacognostic parameters will help for setting standards for crude drugs.

Without specific knowledge of their cellular actions or mechanisms, phytochemicals have been used as poison and in traditional medicine. For example, salicin, having anti-inflammatory and pain-relieving properties, was originally extracted from the bark of the white willow tree and later synthetically produced to become the common, over-the-counter drug, aspirin. The tropane alkaloids of Atropa belladonna were used as poisons, and early humans made poisonous arrows from the plant. In Ancient Rome, it was used as a poison by Agrippina the Younger, wife of Emperor Claudius on advice of Locusta, a lady specialized in poisons, and Livia, who is rumored to have used it to kill her husband Emperor Augustus. The English yew tree was long known to be extremely and immediately toxic to animals that grazed on its leaves or children who ate its berries; however, in 1971, paclitaxel was isolated from it, subsequently becoming an important cancer drug. As of 2017, the biological activities for most phytochemicals are unknown or poorly understood, in isolation or as part of foods. Phytochemical with established roles in the body are classified as essential nutrients. The phytochemical category includes compounds recognized as essential nutrients, which are naturally contained in plants and are required for normal physiological

functions, so must be obtained from the diet in humans. Some phytochemicals are known phytotoxins that are toxic to humans; for example Aristolochic acid is carcinogenic at low doses. Some phytochemicals are antinutrients that interfere with the absorption of nutrients. Others, such as some polyphenols and flavonoids, may be pro-oxidants in high ingested amounts. Non-digestible dietary fibers from plant foods, often considered as a phytochemical, are now generally regarded as a nutrient group having approved health claims for reducing the risk of some types of cancer and coronary heart disease. Eating a diet high in fruits, vegetables, grains, legumes and plant-based beverages has long-term health benefits, but there is no evidence that taking dietary supplements of non-nutrient phytochemicals extracted from plants similarly benefits health. Phytochemical supplements are neither recommended by health authorities for improving health nor approved by regulatory agencies for health claims on product labels.

1.3 Antioxidant activity

Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter .free radicals are fundamentals to any biochemical process and represent an essential part of aerobic life and metabolism .majority if the diseases or disorders are mainly are linked to oxidative stress due to free radicals .the oxidants or radicals are species with very short half of life ,high reactivity and damaging activity towards macromolecules like protein ,DNA and lipids. The leaf extract and seed extracts were evaluated for antioxidant activities by DPPH radical scavenging assay. Among the three accessions with different solvents used, maximum antioxidant activity found ethanol leaf extracts from *Andrographis paniculata*. The present study reveals that these plants are of therapeutic potential due to their high free radical scavenging activity. An antioxidant is a molecule capable of terminating the chain reactions that damage cells by removing free radical intermediates, and inhibit other oxidation reactions by thereby reducing stress responsible for many degenerative disorders. *Andrographis paniculata* Neem, a multipurpose tropical plant is believed to have antioxidant properties. The characteristic secondary metabolites encountered in this plant have considerably enhanced its importance in the arena of medicinal plants. Antioxidants protect your body's cells from free radicals — unstable molecules created during normal cell functions. Pollution, radiation, cigarette smoke, and herbicides also can create free radicals in your body. Free radicals can damage a cell's genetic parts and may trigger

the cell to grow out of control. These changes may contribute to the development of cancer and other diseases. Antioxidants are found in broccoli, brussels sprouts, cabbage, cauliflower, tomatoes, corn, carrots, mangos, sweet potatoes, soybeans, cantaloupe, oranges, spinach, nuts, lettuce, celery, liver, fish oil, seeds, grains, kale, beets, red peppers, potatoes, blueberries, strawberries, and black and green tea. As a rule, dark-colored fruits and vegetables have more antioxidants than other fruits and vegetables.

1.3.1 Determination of antioxidant activity

The antioxidant activity (total antioxidant capacity) of plants and plant extracts can be determined by several in vitro methods. There are two general types of assays widely used for different antioxidant studies. To the first group belong assays that are associated with electron or radical scavenging, including the DPPH assay, Trolox equivalent antioxidant capacity (TEAC) assay, and FRAP assay.

DPPH ASSAY

The DPPH assay is used to predict antioxidant activities by mechanism in which antioxidants act to inhibit lipid oxidation, so scavenging of DPPH radical and therefore determinate free radical scavenging capacity. The method is widely used due to relatively short time required for the analysis. The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical is very stable, reacts with compounds that can donate hydrogen atoms, and has a UV–vis absorption maximum at 515 nm. The method is based on the scavenging of DPPH by antioxidants, which upon a reduction reaction decolorizes the DPPH methanol solution. The assay measures the reducing ability of antioxidants toward the DPPH radical

FRAP TEST

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe2+-TPTZ). The free radical chain breaking takes place through donating a hydrogen atom. At low pH of about 3.6, reduction of Fe3+- TPTZ complex to blue colored Fe^{2+}-TPTZ takes place, which has absorbance at 593 nm. FRAP values of the studied plants varied from 0.36 mg, Trolox equivalent/g dw of sample (*Tinospora cordifolia*) to 18.28 mg, Trolox equivalent/g dw of sample (*Acacia nilotica*). The results obtained are highly reproducible and related linearly with the molar concentration of the antioxidants present.

HYDROGEN PEROXIDE SCAVENGING ACTIVITY

Hydrogen peroxide scavenging activity of TEMPO functionalized dendrimers was determined by monitoring the reduction of H_2O_2.

NITRIC OXIDE ASSAY

Nitric oxide radical scavenging activity of TEMPO functionalized dendrimers was determined by Griess Ilosvay reaction using sodium nitroprusside.

1.4 Antidiabetic activity

Diabetes mellitus is a serious complex multifactorial disorder characterized by hyperglycemia (very high blood glucose level) and glucose intolerance, either due to the relative deficiency in insulin secretion or impaired the effectiveness of insulin's action to enhance glucose uptake. If left untreated, it can lead to severe complications. These complications include hyperlipidemia (abnormal high level of lipid in the blood), oxidative stress, and enzymatic glycation of protein. Many of these conventional drugs have been reported for their inefficiency with prominent adverse side effects. These limitations have largely prompted the exploration of management strategies involving the use of medicinal plants reported to be cost- effective antidiabetic agents with fewer reported side effects. However, the majority of these traditional plants have not been scientifically validated for their efficacy in the treatment of diabetes. Therefore, determination of its efficacy is very important as this plant may play a significant role in the management of diabetes mellitus. Diabetes has been known in India for centuries as "a disease of rich man" but now spread among all masses. Globally, 387 million people are diabetic in 2014. India accounts for 66.84 million diabetics and stands second to China which accounts for 96.28 million. Because of this large number of diabetics, India is to be known as diabetic capital of the world. For evaluation of antidiabetic activity of drugs, in vitro tests can be used as initial screening tools, where the screening of large number of potential therapeutic candidates may be carried out. They might provide useful information on the mechanism of action of therapeutic agents. Therefore, it is necessary to find the integrated treatment of DM by using an alternative medicine such as herbal medicine or using a combination between the synthetic drugs and herbal medicine.

The present study was therefore undertaken to investigate the antidiabetic activity and mechanism of action using various in vitro models designed to stimulate specific antidiabetic targets. *Andrographis paniculata* is an herb native to India belonging to the family Acanthaceae. Because of its extremely bitter taste, it is known as the "King of Bitters" and is a common Ayurveda treatment for various diseases. *Gymnema*

sylvestre is an herb native to tropical Indian forests and has been used in herbal medicine as a treatment for diabetes for nearly two millennia, but there is insufficient scientific evidence to draw definitive conclusions about its efficiency. Eugenia jambolana is the member of Myrtaceae family. It is also known in Hindi as jamun, jambo. *Andrographis paniculata* (Burm. f.) Neem plant originates from India, and has been used for several purposes, primarily preventing diabetes mellitus (DM). Ethanolic extracts of this plant can decrease the blood glucose levels in type 1 DM rats. However, its antidiabetic effect in type 2 DM has not been reported well. *Andrographis paniculata* is an annual herb and widely cultivated in Southeast Asian countries for its medicinal use. In recent investigations*, A. paniculata* was found to be effective against Type 1 diabetes mellitus (Type 1 DM). Here, we used a non-genetic out-bred Sprague-Dawley rat model to test the antidiabetic activity of *A. paniculata* against Type 2 diabetes mellitus (Type 2 DM). Proton Nuclear Magnetic Resonance (1H-NMR) spectroscopy in combination with multivariate data analyses was used to evaluate the *A. paniculata* and metformin induced metabolic effects on the obese and obese–diabetic (obdb) rat models. Compared to the normal rats, high levels of creatinine, lactate, and allantoin were found in the urine of obese rats, whereas, obese-diabetic rats were marked by high glucose, choline and taurine levels, and low lactate, formate, creatinine, citrate, 2-oxoglutarate, succinate, dimethylamine, acetoacetate, acetate, allantoin and hippurate levels. Treatment of *A. paniculata* leaf water extract was found to be quite effective in restoring the disturbed metabolic profile of obdb rats back towards normal conditions. This study shows the anti-diabetic potential of *A. paniculata* plant extract and strengthens the idea of using this plant against the diabetes. Further classical genetic methods and state of the art molecular techniques could provide insights into the molecular mechanisms involved in the pathogenesis of diabetes mellitus and anti-diabetic effects of *A. paniculata* water extract.

1.4.1 Taxonomical classification (*Andrographis paniculata*; green chiretta)

Kingdom: Plantae-- planta, plantes, plants, vegetal

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnolioophyta

Class: Magnoliopsida

Order: Lamiales

Family: Acanthacea

Genus: Andrographis

Species: *Andrographis paniculata*

1.5 Objectives

The main objectives of the study is to

• screen the phytochemical present in the aqueous extract *of Andrographis paniculata* leaves.

• compare the phytochemical analysis of aqueous leaf extract of *Andrographis paniculata* using different solvent ethanol and chloroform

• screen the Antioxidant activity of aqueous extract of Andrographis paniculata leaves.

• screen the Antidiabetic activity of aqueous extract of *Andrographis paniculata* leaves.

2. Review of literature

The importance of medicinal plant in drug development is known to us and humans have used them for different diseases from the beginning of human history. Traditional folk treatment from wild plants has always guided researchers to search for novel medications to develop healthy life for humans and animals. In addition, some medicinal plants are still obscured within the plant which need to be scientifically evaluated. The universal role of plants in the treatment of disease is exemplified by their employment in all the major systems of medicine. There is a great wealth of knowledge concerning the medicinal and other properties of plants that is transmitted from generation to generation by tribal societies. The use of modern isolation techniques means that new plant drugs usually find their way into medicine as purified substances rather than in the form of galenical preparations.

The use of single pure compounds, including synthetic drugs, is not without its limitations, and in recent years there has been an immense revival in interest in the herbal system of medicine, which relies heavily on plant sources. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value which have yet to be discovered; large numbers of pants are constantly being screened for their possible bioactivity (Devans et al., 2010).

2.1 *Andrographis paniculata*

Andrographis paniculata is commonly known as creat or green chiretta belongs to the family Acanthaceae. It is also known as Kalmegh and popularly called as King of bitters. It was widely used in the traditional system of medicines such as Siddha system, Ayurvedic system and Chinese medicine for the regular treatment of a common cold. Some of the reported health benefits are Antidiabetic, Antipyretic, Hepatoprotective, Antioxidant, Antimalarial, Anti-cancer, Antityphoid, Anti-diarrhoea, Antioedema, etc. Whole plant possesses medicinal property and leaves are explicitly used for therapeutic care. The plant contains four major diterpenoids such as andrographolide, neoandrographolide,14- deoxyandrographolide, 14-deoxy-11,12-didehydroandrographolide and also includes other phytoconstituents such as flavonoids sesquiterpene lactones, etc. Andrographolide is found as major constituent in the leaves and reported for a wide variety of pharmacological activities. The present analysis also illustrates the pharmacological and phytochemical characteristics of *Andrographis paniculata* from which researchers may get benefited for further qualitative research.

Andrographis paniculata is an annual herbaceous plant native to India, Sri Lanka, Pakistan, and Indonesia and has a spread in China, Thailand, Mauritius, East and West Indies (Mishra, Sangwan, & Sangwan, 2007).

Usually cultivated by seeds, grows well in any type of soil with proper organic matter. The plant can be cultivated in plain land, grounds, hills, moist habitat, farms, and roadsides. It can also be grown through tissue culture. Andrographolide will be more in leaves during flowering. Good yield can be got within 90-100 days. The rainy season is the best time to cultivate the plant (Mishra et al., 2007; Nitave et al., 2016).

Twenty diterpenoids, ten flavonoids, and sesquiterpene lactone were reported from AP. The major diterpenoids are andrographolide, 14-deoxyandrographolide, neoandrographolide, 14-deoxy-11, 12-didehydroandrographide, isoandrographolide. Ethyl acetate soluble fractions of Ethanol or methanol extract contain 2'-O-β-D-glucopyranoside, 5, 2'-dihydroxy-7, 8 dimethoxyflavone, 7-O- methylwogonin, 5-hydroxy-7, 8-dimethoxyflavone, 2'-hydroxy-5, 7 ,8 trimethoxyflavone, wightin, 5,7,8,2’ tetramethoxyflavone, 5-hydroxy-7, 8- dimethoxyflavone, andrographolide A, 5, 2'-dihydroxy-7, 8-dimethoxy flavone, andrographolide B, and rographolide C, from roots β-sitosterol, β-daucosterol and lupeol, triacylglycerols, monogalactosyl diacylglycerols from pods were isolated. Andrographis acid a diterpenoid was reported (Chao & Lin, 2010).

Andrographolide can be isolated and purified by column chromatography methods, hydrotropic microwave-assisted extraction, and soxhlet extraction. Its purity is determined by high-performance liquid chromatography, thin-layer chromatography, UV absorption spectrum, liquid chromatography-mass spectrometry, and differential scanning calorimetry (Jadhao, 2013).

2.2 Phytochemical analysis

Plant-produced chemical compounds or phytochemicals like alkaloids, glycosides, flavonoids, volatile oils, tannins, resins have been used in a wide range of commercial and industrial applications such as flavors, aromas and fragrances, enzymes, preservatives, cosmetics, biobased fuels and plastics, natural pigments and bioactive compounds. The research on phytochemicals and use of phytochemicals is increasing more because of the harmful side effects of the synthetic compounds (M. Sharma and R. Sharma [76]).

The aerial parts of AP have been described for its in numerous 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. The highest amount of andrographolide, a major bioactive compound of AP, 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 ($CHCl_3$), and dichloromethane from the whole plant, leaves, aerial parts, stems, and roots.

Extraction procedure of bioactive compounds of AP from MeOH extracts, for example, is shown in Figure 2. In this extraction procedure, whole plant material of AP (11.5 kg) was shade dried, ground, extracted with methanol (10 L × 6) under reflux for 8 h, and filtered to give residue. 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 AP have reported more than 55 ent-labdane diterpenoids, 30 flavonoids, 8 quinic acids, 4 xanthones (Qader SW, Abdulla MA, Chua LS, Sirat HM, Hamdan S Int J Mol Sci. 2012; 13).

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. Medicinal plants have been used to treat illness and disease

for thousands of years. Even now they are economically important, being used in the pharmaceutical, cosmetic, perfumery, and food industries. Screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic use (Gowdhami.M et al., 2014).

The demand of AP is greatly increased in the past few years for its overwhelming therapeutic potentials. Available data on AP also clearly expresses a broad spectrum of pharmacological properties of this plant. Due to possessing extensive pharmacological activities, the AP can be safely regarded as one of the modern catholicons. However, the investigated pharmacological activities of AP need validation through the clinical study. The use of medicinal plants has been evidenced since ancient times for the treatment of a wide range of illnesses, and they have become increasingly important in healthcare. Although the use of phytomedicines was based on empiric experience in the past, nowadays is increasingly based on scientific evidence regarding their chemical composition and associated medicinal properties (Nychas et al., 2003).

It is well known that many medicinal plants with similar morphology and the same folk name among species within the same genus can be misidentified and intentionally or accidentally be substituted in commercial products during the manufacturing process, and may result in the loss of efficacy and safety. The authentication of biological species is usually performed by experienced taxonomy however, morphological identification can be limited by the absence of different phenotypic characteristics. Furthermore, herbal products are available in the marked as their processed forms, such as herbs (i.e leaf powder and crushed material), herbal material (i.e. essential oils), herbal preparations (i.e. extracts) and finished herbal products (i.e. capsules and tablets), and consequently morphological identification is quite challenging2,3. Therefore, phytochemical studies may provide a useful tool for the authentication and discrimination between similar plant species. The traditional medicine involves the use of different plant extracts or the bioactive constituents. This type of study provides the health application at affordable cost. Secondary metabolites are responsible for medicinal activity of plants. Qualitative phytochemical analysis of medicinal plants confirm the presence of various phytochemicals like saponins , terpenoids , steroids , anthocyanins h coumarins , fatty acids , tannins , leucoanthocyanins , and emodins . The results suggest that the phytochemical properties for curing various ailments and

possess potential antioxidant and leads to the isolation of new and novel compounds (N. Savithramma et al., 2014).

2.3 Antioxidant activity

Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter .free radicals are fundamentals to any biochemical process and represent an essential part of aerobic life and metabolism .majority if the diseases or disorders are mainly are linked to oxidative stress due to free radicals .the oxidants or radicals are species with very short half of life ,high reactivity and damaging activity towards macromolecules like protein, DNA and lipids.(Suparna Deepak et al .,2014).

The leaf extract and seed extracts were evaluated for antioxidant activities by DPPH radical scavenging assay. Among the three accessions with different solvents used, maximum antioxidant activity found ethanolic leaf extracts from *Andrographis paniculata.* The present study reveals that these plants are of therapeutic potential due to their high free radical scavenging activity (Kavitha Vijayaraghavan et al 2013).

Antioxidants can protect against the cell damage that free radicals cause, known as oxidative stress.

Activities and processes that can lead to oxidative stress include Trusted Source: Mitochondrial activity, excessive exercise, tissue trauma, due to inflammation and injury, ischemia and reperfusion damage, consumption of certain foods, especially refined and processed foods, trans fats, artificial sweeteners, and certain dyes and additives, smoking, environmental pollution, radiation, exposure to chemicals, such as pesticides and drugs, including chemotherapy, industrial solvents, ozone. Such activities and exposures can result in cell damage. This, in turn, may lead to: an excessive release of free iron or copper ions, an activation of phagocytes, a type of white blood cell with a role in fighting infection, an increase in enzymes that generate free radicals , a disruption of electron transport chains All these can result in oxidative stress. The damage caused by oxidative stress has been linked to cancer, atherosclerosis, and vision loss. It is thought that the free radicals cause changes in the cells that lead to these and possibly other conditions. Flavonoids, flavones, catechins, polyphenols, and phytoestrogens are all types of antioxidants and phytonutrients, and they are all found in plant-based foods. Each antioxidant serves a different function and is not interchangeable with another. This is why it is important to have a varied diet (Hridhya K V et al., 2017).

There is an increasing trend to replace synthetic antioxidants, which are of safety concern, with the natural antioxidants available from plant extracts or isolated products of plant origin. Present study was undertaken to determine phytochemical content and ROS scavenging inhibitory activity of 11 medicinal plants from Western Ghats of India in order to evaluate their potential as a natural antioxidative source. The plants are used traditionally for diseases such as bronchitis, diabetes, heart diseases and asthma for several years. The antioxidant activities were determined by in vitro assays to compare their antioxidant effects. These include inhibition of DPPH (1,1-diphenyl-2-picrylhydrazyl), Trolox equivalent antioxidant capacity (TEAC) using ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) as an oxidant and FRAP (Ferric reducing antioxidant power). Total phenolic and flavonoid contents were also determined (Tripathi R et al.,2012).

Three methods widely employed in the evaluation of antioxidant activity, namely 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, static headspace gas chromatography (HS-GC) and beta-carotene bleaching test (BCBT), have been compared with regard to their application in the screening of plant extracts. The strengths and limitations of each method have been illustrated by testing a number of extracts, of differing polarity, from plants of the genus Sideritis, and two known antioxidants (butylated hydroxytoluene and rosmarinic acid). The sample polarity was important for the exhibited activity in the BCBT and HS-GC methods but not for the DPPH method. The complex composition of the extracts and partition phenomena affected their activity in each assay. The value of the BCBT method appears to be limited to less polar samples. Although slow, the HS-GC method is preferable for assessing the antioxidant inhibitory properties on the formation of unwanted secondary volatile products. Being rapid, simple and independent of sample polarity, the DPPH method is very convenient for the quick screening of many samples for radical scavenging activity (Lissi E et al., 2002).

2.4 Antidiabetic activity

Diabetes mellitus, one of the major public health problems worldwide, is a metabolic disorder of multiple etiologies distinguished by a failure of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism as a result of defects in insulin secretion and/or insulin action. According to International Diabetes Federation (IDF) report, elevated blood glucose is the third uppermost risk factor for premature mortality, following high blood pressure and tobacco use globally. Cardiovascular

diseases, neuropathy, nephropathy, and retinopathy are among the major risks that are associated with diabetes. These chronic complications may lead to hardening and narrowing of arteries (atherosclerosis) that could advance to stroke, coronary heart disease, and other blood vessel diseases, nerve damage, kidney failure, and blindness with time (Rubin R et al.,2012)

In 2015, according to IDF report, 415 million (8.8%) adults (aged 20–79) worldwide were estimated to have diabetes; this number is expected to rise to 642 million (10.4%) by 2040 or one adult in ten people. An estimated 14.2 million adults aged 20–79 had diabetes in the Africa Region that represents a regional prevalence of 3.2% (2.1–6.7%) in 2015, which can be projected to 3.7% (2.6–7.3%; 34.2 million) by 2040. South Africa (2.3 million), Democratic Republic of Congo (1.8 million), Nigeria (1.6 million) and Ethiopia (1.3 million) are among the highly populated African countries containing the highest number of people living with diabetes (Rajpathak S et al.,2015).

Diabetes mellitus can be managed by diet, physical exercise, and modern drugs (insulin and/or oral hypoglycemic drugs such as sulfonylureas and biguanides). Different extracts from medicinal plants have also been used traditionally to manage diabetes globally, and these are considered as relatively inexpensive, less toxic and with relatively little or no side effects. There are also medicinal plants that contain some toxic constituents such as the cytotoxic anti-cancer plant-derived drugs, digitalis; however, the side effects of the phytotherapeutic agents are less common compared with synthetic drugs.

Management of diabetes without any side effect is still a challenge and the available modern antidiabetic agents produce serious side effects such as hypoglycemia (Sulphonylureas), lactic acidosis and folate and B12 malabsorption (Metformin), gastrointestinal symptom (Acarbose), weight gain (Sulphonylureas and Thiazolidinediones), and edema (Thiazolidinediones). Hence, the search for safer and more effective hypoglycemic agents has continued (Koski RR et al 2006).

Diabetes in rats was induced by a single dose of 5% alloxan monohydrate (125 mg/kg, i.p.) after 24 h fasting. Induction of diabetes was confirmed after a week of alloxan treatment by estimation of fasting blood glucose level. Only those rats with blood glucose level between 200–300 mg/dl were included in the study. These rats were further divided into seven groups (I-nondiabetic control; II-diabetic control; III-Aqueous extract, IV-Ether extract, V-Methanolic extract, VI-Glibenclamide, VII-Glibenclamide + Aqueous extract) of six rats each. Groups III–V were subgrouped (IIIA, IIIB, IVA, IVB,

VA, VB). Groups I and II (control) received comparable volume of NSS. Groups III–V received lower and higher daily doses of AE, EE, and ME at a rate of 50 and 100 mg/kg p.o., respectively, once daily for 4 weeks. VIth group was administered hypoglycemic drug glibenclamide (5 mg/kg, p.o.) once daily for 4 weeks and group VII was administered daily dose of glibenclamide (50 mg/kg) and 100 mg/kg AE, p.o., respectively. The blood glucose levels were measured by glucometer on day 0, 1, 5, 7, 14, and 28. The blood samples were collected from tail vein puncture and blood glucose levels were analyzed (R. S. Kujur et al 2010).

The plant (leaf) is use traditionally by the Ibibios of Akwa Ibom State, Nigeria in the treatment of various ailments such as rheumatism pain, inflammation and diabetes. Ethnopharmacological and scientific reports on this plant is scarce. In this study , we investigated the effect of ethanolic leaf extract of Paniculata maximum on alloxan induced diabetic rats during single and repeated administrations to observe acute and chronic effects of the extract on blood glucose levels of the diabetic rats in bids to confirm its ethnobotanical uses in the management of diabetes (Bassey S. Antia et al 2010).

Table 1. Different vernacular names of *Andrographis paniculata* around the globe and India.

Language	**Names**
Scientific names	*Andrographis paniculata*
Name in various global languages	
French	
German	
English	Green chiretta
Name in various Indian languages	
Sanskrit	Bhunimba
Hindi	Hara chirayata
Telugu	Nelavemu
Marathi	Kalamegha
Kannada	Nelabevu
Gujarati	Kariyatu
Malayalam	Kiriyatta
Tamil	Nilavempu

3. Hypothesis

The current research work is based on the following hypothesis

1) Plant extracts of *Andrographis paniculata* is rich in secondary metabolites.
2) The extracts shows antidiabetic activities.

4. Materials and Methods

4.1 Study area

Kerala state covers an area of 38,863 km^2 with a population density of 859 per km^2 and spread across 14 districts. The climate is characterized by tropical wet and dry with average annual rainfall amounts to 2,817 ± 406 mm and mean annual temperature is 26.8°C (averages from 1871-2005; Krishnakumar et al., 2009). Maximum rainfall occurs from June to September mainly due to South West Monsoon and temperatures are highest in May and November.

4.2 Sample collection

The leaves of *Andrographis paniculata* were identified and collected based on interaction with farmers and selected for morphological characterization, phytochemical characterization and anticancer properties from various districts across Kerala State, India. Flowers collected from different varieties and were thoroughly cleaned using double distilled water. The samples were cut into small pieces and dried in hot air oven at 60ºC for 48 hrs, powdered using a kitchen blender (Prestige Nakshatra plus, Prestige industries Mumbai) and later stored in air tight polyethylene zipper bag for analysis.

4.3 Sample preparation

The collected leaves of *Andrographis paniculata* were washed thoroughly thrice under running tap water and once with distilled water. Then the washed leaves were shade dried for 5 – 7 days. The dried leaves were then powdered using a kitchen blender and then it is kept in a tight container. Powdered leaves more subjected to extracted with Ethanol and chloroform solvent by the use of Soxhlet apparatus. Then these ethanol and chloroform extracted samples is used for phytochemical analysis

4.4 Preparation of plant extract

10 g dried leaf powder of *Andrographis paniculata* were taken and mixed with 100 ml of ethanol solvent using Soxhlet apparatus. The same procedure was repeated for chloroform extract.

4.5 Phytochemical analysis

The phytochemical testing is used for extraction, screening and identification of medically useful compounds in the given sample because the phytochemicals are promoted for the prevention and treatment of many health conditions, such as cancer, heart disease, diabetes and high blood pressure.

The present study describes the phytochemical activity of *Andrographis paniculata*. For the present investigation, two samples of *A. paniculata* extracts, obtained by extraction in “Chloroform and Ethanol”. Alkaloids, tannins, terpenoids, glycosides, flavonoids, saponins and phenols of different plant extracts are tested as per standard protocols (Ogbonna et al ., 2016).

4.5.1 Test for alkaloids (Mayer’s test)

The extracts were dissolved individually in dilute hydrochloric acid (HCL) and filtered. The filtrate treated with Mayer’s reagent, formation of yellow color precipitate indicates the presence of alkaloids.

4.5.2 Test for phenols (ferric chloride test)

Extract were treated with 3-4 drop of ferric chloride ($FeCl_3$) solution .formation of bluish black colour indicates presence of phenols.

4.5.3 Test for reducing sugars (Fehling’s test)

To 1ml of the extract added few drops of Fehling's reagent and the mixture was boiled in a boiling water bath for 10 minutes and observed for the appearance of blue colour.

4.5.4 Saponins (Frothing/Foam test)

Add 0.5ml of filtrate with 5ml of distilled water and shake well. Persistence of frothing shows presence of Saponins.

4.5.5 Test for flavonoids (alkaline reagent test)

2ml of 2.0% NaOH mixture was mixed with 1 ml of crude plant extract. Concentrated yellow colour will be produced and when two drops of diluted acid was added to the mixture, the colour disappears, which indicates the presence of flavonoid.

4.5.6 Test for steroids

2ml of chloroform and 0.2 ml of concentrated H_2SO_4 were added to 5 ml of plant extract red color appears in lower layer indicating the presence of steroids.

4.5.7 Test for amino acids and proteins (Ninhydrin test)

To the extract, 0.25 % w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid and protein.

4.5.8 Test for tannins

Two drops of 5% $FeCl_3$ was added to 1 ml of plant extract .the appearance of a dark green or blue precipitate indicate the presence of tannins.

4.5.9 Test for phytosterols

1 ml of the plant extract was treated with 2 ml of chloroform and few drops of acetic anhydride were added. To that mixture added equal amount of concentrated sulphuric acid was added. The formation of bluish green colour indicates the presence of phytosterols.

4.5.10 Test for glycosides

Few drops of $FeCl_3$ and concentrated H_2SO_4 were added to a solution of plant extract mixed with equal volume of glacial acetic acid. A reddish brown coloration at the junction of two layers and the bluish green colour in the upper layer indicates presence of glycosides.

4.6 Antioxidant activity

The antioxidant activity (total antioxidant capacity) of plants and plant extracts can be determined by several in vitro methods. Antioxidant activity of aqueous leaf extracts of *Andrographis paniculata* were determined by these methods.

- DPPH ASSAY
- FRAP TEST
- NITRIC OXIDE ASSAY
- HYDROGEN PEROXIDE ASSAY

Antioxidant activity of plant samples are analyzed using standard protocols.

4.6.1 Assay of DPPH radical scavenging activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. 1ml sample of various concentrations (10 - 50µg/ml) of plant extract (PE) was added to 80% methanolic solution of DPPH (0.2mM) and shaken vigorously. The mixtures were incubated in the dark for 15 min at room temperature. Ascorbic acid was used as standard and methanol served as blank. The solution without sample was served as control. The absorbance of the samples was recorded at 518nm by using a spectrophotometer. The experiment was expressed as the percent inhibition of free radicals by the sample and was calculated using the following.

$$\text{DPPH activity}(\%) = \frac{(C-S)}{(C)} \times 100$$

Where , C =Absorbance of control, S = Absorbance of sample

4.6.2 FRAP test

The method is based on the reduction of Fe^{3+} TPTZ complex (colorless complex) to Fe^{2+} -tripyridyltriazine (blue colored complex) formed by the action of electron donating antioxidant at PH. This reaction is monitored by measuring the change in absorbance at 593 nm. The Ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 ml TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in the proportion of 10:1:1 at 37°C. Freshly prepared working FRAP reagent was pipetted using 1-5 ml variable micropipette (1ml) and mixed with 10 - 50µl of the appropriately diluted plant sample and mixed thoroughly. The absorbance at 593 nm was recorded against a reagent blank (1ml FRAP reagent+ distilled water) after 30 min incubation at 37°C.

4.6.3 Nitric oxide assay

The extracts were prepared from a 10 mg/mL ethanol crude extract. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations of the ethanol extracts (10−50 µg/mL) and incubated at 25°C for 180 mins. The extract was mixed with an equal volume of freshly prepared Griess reagent.

Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The absorbance was measured at 546 nm using a SpectraMax Plus UV-Vis microplate reader .

percentage nitrite radical scavenging activity:

$$\text{Nitric Oxide } (\%) = \frac{\left(A_{control} - A_{test}\right) X100}{A_{test}} \qquad (2)$$

Where,

$A_{control}$ = Absorbance of control reaction

A_{test} = Absorbance in the presence of the samples of extract.

4.6.4 Hydrogen peroxide assay

The radical scavenging activity of methanolic, ethanolic and aqueous extracts of the plants to scavenge hydrogen peroxide (H_2O_2). 1ml sample of various concentrations

(10-50 µg/ml) of plant extract (PE) were added to 1ml of H_2O_2 (40 mM) prepared in (50 mM, pH-7.4) phosphate buffer. The test samples were incubated for 10 min at room temperature. The absorbance was measured at 230 nm (Thermo Scientific UV 1). Phosphate buffer without H_2O_2 was used for blank and hydrogen peroxide solution without extract served as control. Ascorbic acid was used as a standard.

Hydrogen peroxide scavenging activity was calculated by following formula:

$$\text{Hydrogen peroxide scavenging activity (\%)} = \frac{(C - T)}{(C)} \times 100$$

Where, C = absorbance of control T= absorbance of test sample

4.7 Antidiabetic activity

4.7.1 Alpha amylase activity

Starch azur (2mg) was suspended into tubes containing 0.2 ml of 0.5 M Tris HCL buffer (PH 6.9) and 0.1M $CaCl_2$. Tubes were boiled for 5 mts then incubated at 37°C for 5 mts. Plant extract (0.2ml) in each tube at different concentration (10, 20…50 µg) of Dimethyl sulfoxide (DMSO). PPA dissolve in Tris-HCL buffer forming a concentration of 2 units / ml in another tube. 0.1 ml of PPA solution was added to six different concentration of DMSO tube. Reaction carried out at 37°C for 10mts . Reaction stopped by adding 0.5ml Acetic acid in each tube. Reaction mixture centrifuge at 3000rpm for 5mts at 4°C. Absorbance measured at 595nm.

$$\%\,\text{inhibition} = \left(1 - \frac{A_{540\,\text{sample}}}{A_{540\,\text{negative control}}}\right) \times 100$$

4.8 Statistical analysis

The results were analyzed and descriptive statistics were done using SPSS 12.0 (SPSS Inc., an IBM Company, Chicago, USA) and graphs were generated using Sigma Plot 7 (Systat Software Inc., Chicago, USA).

5. Results and discussion

5.1 Phytochemical analysis

In the present study the comparative analysis of phytochemical constituents occurring in the ethanolic and chloroform extraction of *Andrographis paniculata* were analyzed

qualitatively by phytochemical screening. The presence or absence of colour change indicates positive and negative results. The results of comparative study of phytochemical screening of aqueous extract of *Andrographis paniculata* are shown in The presence of this phytochemical compounds shows the medical value of the *Andrographis paniculata*. The Ethanol leaf extracts of *Andrographis paniculata* contain alkaloids, flavonoids, saponins, Tannins, reducing sugar, phenols, steroids, glycosides and Protein. Phytosterols is absent in the Ethanol extract.

The Chloroform leaf extract of *Andrographis paniculata* confirmed the presence of phytosterols, steroids and protein.

HENCE PHYTOCHEMICAL COMPOUNDS ARE DOMINANT IN ETHANOL EXTRACT OF "*Andrographis paniculate*". So the remaining procedure is done in Ethanol extract of *Andrographis paniculata*.

5.2 Evaluation of invitro antioxidant activity

5.2.1 Diphenyl-Picryl Hydrazyl Assay of ethanol extracts of *Andrographis paniculata*

The DPPH assay is used to predict antioxidant activities by mechanism in which antioxidants act to inhibit lipid oxidation, so scavenging of DPPH radical and therefore determinate free radical scavenging capacity. The method is widely used due to relatively short time required for the analysis.

5.2.2 Ferric reducing antioxidant power (FRAP) test

The method is based on the reduction of Fe^{3+} TPTZ complex (colorless complex) to Fe^{2+} -tripyridyltriazine (blue colored complex) formed by the action of electron donating antioxidant at PH. The results obtained are highly reproducible and related linearly with the molar concentration of the antioxidants present.

5.2.3 Nitric oxide radical scavenging assay

Nitric oxide radical scavenging activity of TEMPO functionalized dendrimers was determined by Griess Ilosvay reaction using sodium nitroprusside. The absorbance was measured at 546 nm using a SpectraMax Plus UV-Vis Spectrophotometer .

5.2.4 Hydrogen peroxide scavenging activity

The scavenging activity of extract towards hydrogen peroxide radicals was determined by the modified method of Dehpour. Solution of hydrogen peroxide (40Mm) was prepared in phosphate buffer pH 7.4 and its concentration was determined by measuring the absorbance at 230nm using UV spectrophotometer. 0.1mg/ml of the extract was added to hydrogen peroxide solution and absorbance measured at 230nm

using UV spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract and standard compound.

5.3 Evaluation of invitro antidiabetic activity

5.3.1 Invitro Alpha amylase inhibitor

The alpha-amylase inhibitor is a protein family which inhibits mammalian alpha-amylases specifically, by forming a tight stoichiometric 1:1 complex with alpha-amylase. This family of inhibitors has no action on plant and microbial alpha amylases. A crystal structure has been determined for tendamistat, the 74-amino acid inhibitor produced by *Streptomyces tendae* that targets a wide range of mammalian alpha-amylases. The binding of tendamistat to alpha-amylase leads to the steric blockage of the active site of the enzyme. The crystal structure of tendamistat revealed an immunoglobulin-like fold that could potentially adopt multiple conformations. Such molecular flexibility could enable an induced-fit type of binding that would both optimise binding and allow broad target specificity.

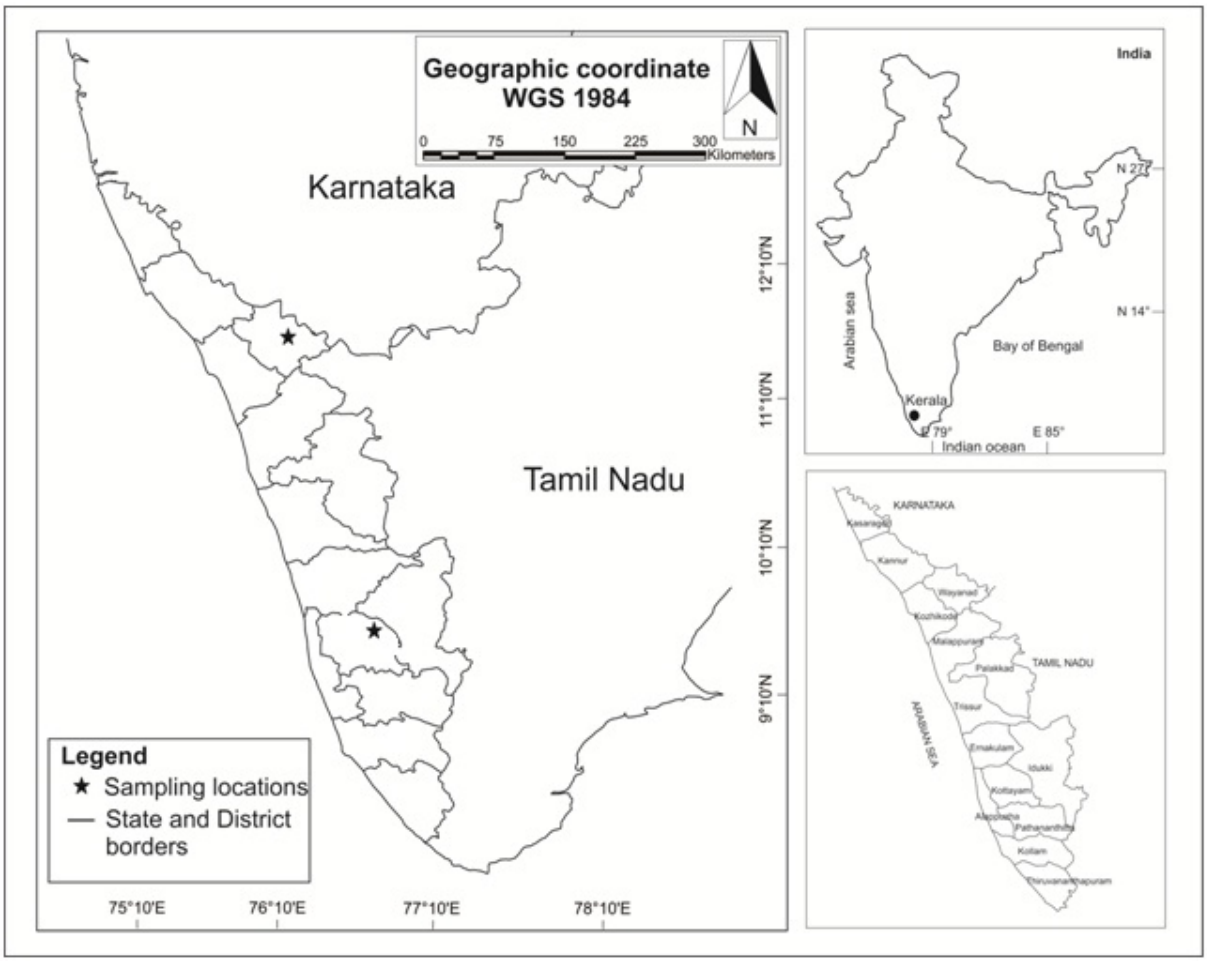


Figure 1. Map of Kerala showing the sample collection point.

Figure 2. Description of *Andrographis paniculata* a), c) and d) plant with flowers, b) and d) flowers. Photo courtesy: Wikipedia .

Table 2. Phytochemical analysis of the Ethanol and Chloroform extraction of *Andrographis paniculata*

COMPOUNDS	ETHANOL	CHLOROFORM
Alkaloids	+	–
Flavonoids	+	–
Saponins	+	–
Tannins	+	–
Reducing sugar	+	–
Phenols	+	–
Steroids	+	+
Phytosterol	–	+
Glycosides	+	–
Protein	+	+

Table 3. Percentage scavenged by ethanol leaf extract of *Andrographis paniculata*

SAMPLE CONCENTRATION(MG/ML)	**ABSORBANCE AT 517nm**	**% SCAVENGED**
Control	0.571	
10	0.535	6.30%
20	0.520	8.90%
30	0.512	10.30%
40	0.413	27.67%
50	0.253	55.69%

Table 4. Percentage scavenged by ethanol leaf extract of *Andrographis paniculata*

SAMPLE CONCENTRATION(MG/ML)	ABSORBANCE AT 593nm	% SCAVENGED
Control	0.9639	
10	0.7295	24.3%
20	0.5581	42.0%
30	0.5577	42.1%
40	0.3357	65.1%
50	0.0778	91.9%

Table 5. Percentage scavenged by ethanol leaf extract of *Andrographis paniculata*

SAMPLE CONCENTRATION (mg/ml)	**ABSORBANCE AT 540nm**	**% SCAVENGED**
Control	0.430	
10	0.383	10.9%
20	0.332	22.7%
30	0.320	25.5%
40	0.271	36.9%
50	0.171	60.2%

Table 6. Percentage scavenged by ethanol leaf extract of *Andrographis paniculata*

SAMPLE CONCENTRATION (mg/ml)	ABSORBANCE AT 230nm	% SCAVENGED
Control	0.0349	
10	0.0250	28.3%
20	0.0195	44.1%
30	0.0081	97.6%
40	0.0068	98.0%
50	0.0047	98.6%

Table 7. Alpha amylase inhibition

SAMPLE CONCENTRATION (mg/ml)	ABSORBANCE AT 540nm
Control	0.082
10	0.064
20	0.095
30	0.11
40	0.23
50	0.45

Figure 3. Description of a) *Andrographis paniculate* leaves in powder form b), c) and d) preparation of ethanol and chloroform extract, e) phytochemical analysis using ethanol as solvent, f), phytochemical analysis using e as solvent.

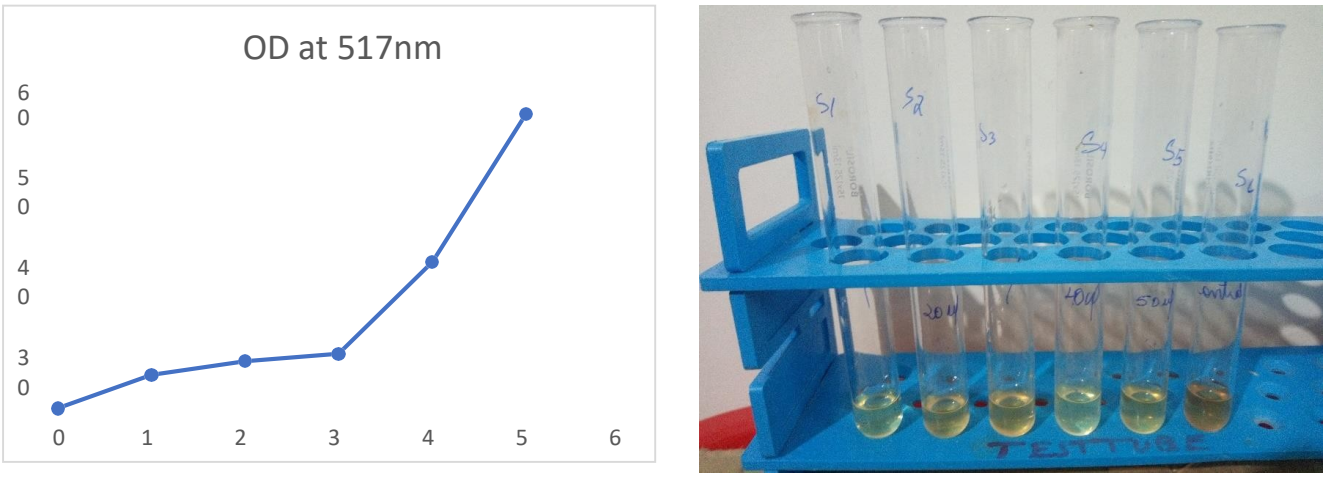


Figure 4. DPPH Assay.

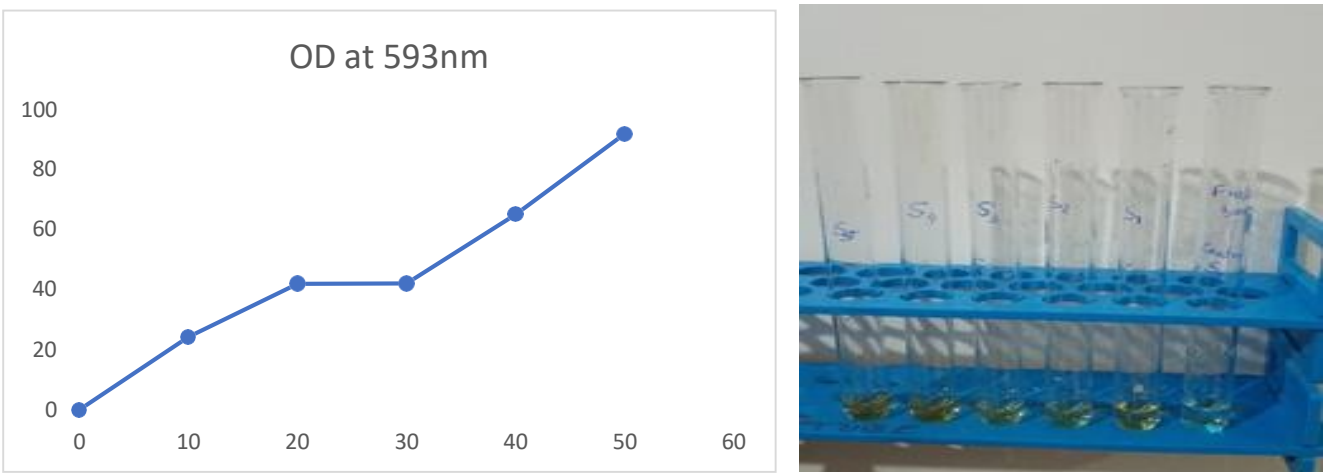


Figure 5. FRAP test

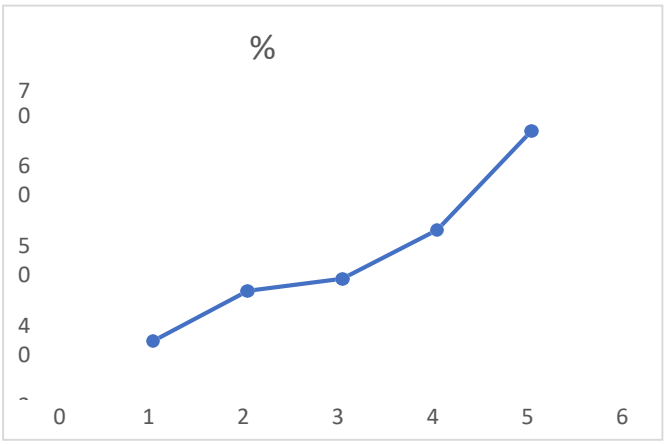
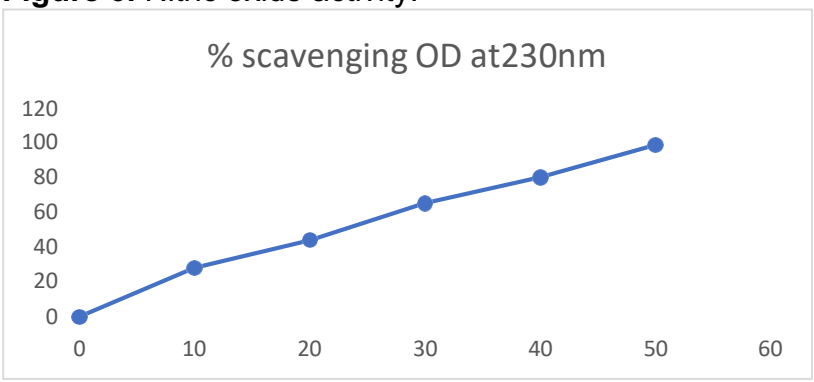
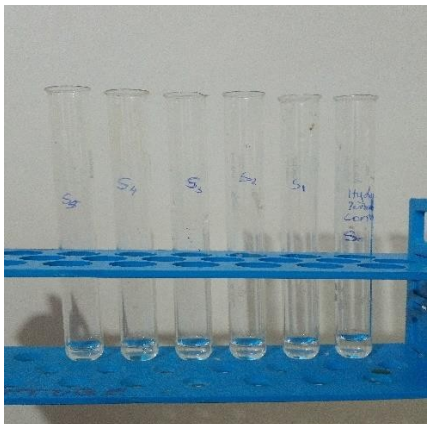


Figure 6. Nitric oxide activity.





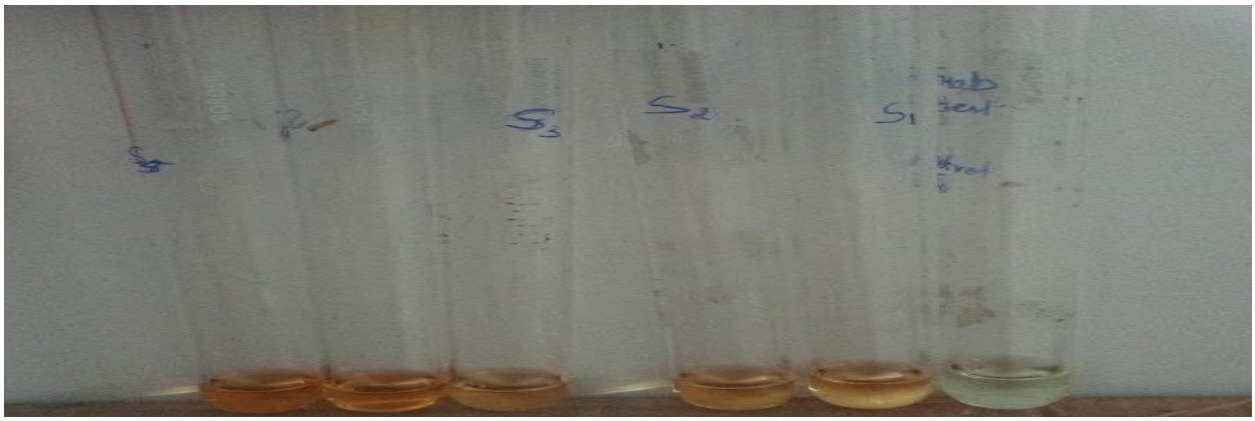


Figure 7. Hydrogen peroxide activity.

6. Conclusions

In the present study, a comparative analysis was performed on the Ethanol and Chloroform leaf extracts of *Andrographis paniculata*. The leaf extracts of plants were prepared. The Ethanol and Chloroform is used as solvent for extraction process. The phytochemical analysis of the plant extracts were carried out.

Among the solvents the Ethanol shows more positive to phytochemical compounds. Hence for further procedure Ethanol extract of *Andrographis paniculata* is taken. The antioxidant activity is determined by DPPH assay, FRAP test, Nitric oxide assay, hydrogen peroxide assay. The antidiabetic activity of *Andrographis paniculata* was determined by Alpha amylase activity. In the present study invented *Andrographis paniculata* helps to stimulating the immune system. It might also prevent influenza viruses from binding to cells in the body. This investigation using *Andrographis paniculata* will focus on cultivation condition of plant production and also about the health benefits on human consuming *Andrographis paniculata*.

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Index

	W	World Health Organization	8
willow tree	9, 10, 17, 24, 28, 41		