Evaluation of Antipyretic and Antimicrobial efficacy of *Andrographis paniculata* in different formulations

By

DEEPIKA E

(17PZO003)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women Coimbatore – 641 043

In Partial Fulfilment of the Requirements for the Degree of

Master of Science in Zoology

April, 2019

19/4/2019

Signature of the

Head of the Department

Sandhya.K.S
19.4.2019

Signature of the

Supervisor

ACKNOWLEDGEMENT

First and foremost, I owe my sincere and humble gratitude to **GOD ALMIGHTY** for the grace and abundant blessings showered for the successfulcompletion of this study.

I wish to record my sincere thanks and gratitude to **Shri. Dr. P.R. Krishnakumar,** Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for providing me the opportunity to undertake this investigation in this esteemed institution.

I record my gratitude and heartfelt thanks to **Dr. (Mrs.) Premavathy Vijayan, M.Sc., M.Sc., M.Ed., Dip.Spl.Edu (U.K), Ph.D., (Avinashilingam),** Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women,Coimbatore for the facilities provided to carry out the project work.

My sincere thanks to **Dr. (Mrs) S. Kowsalya, M.Sc., M.Phil., Ph.D.** Registrar, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for her motivation and help in the conduct of the study.

I am greatly indebted and thankful to **Dr. (Mrs) P.R. Padma, M.Sc., Ph.D.Adv.Dip.,**Dean, School of Bioscience, Avinashilingam Institute for Home Scienceand Higher Education for Women, Coimbatore for her continued interest and constantsupport in the conduct of project work.

I express my deep sense of gratitude to **Dr. (Mrs) N.Krishnaveni, M.Sc., B.Ed., Ph.D., NET** Professor and Head, Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for theconstant support given during the period of investigation.

I express my thanks and gratitude to my beloved guide **Dr. (Mrs) K. S. Santhy, M.Sc., M.Phil., Ph.D., MCA., PGDIBI.,** Professor, Department of Zoology, Avinashilingam Institute for Home Science andHigher Education for

Women, Coimbatore for her incessant guidance, constant support, valuable help, patience and encouragement shown throughout the period of the study.

I solemnly thank **Siddha Regional Research Institute**, Poojapura, Tiruvananthapuram for supplying the stuffs related to the study

I take this opportunity to thank the **Staff members** and **Lab Assistants** in the Department of Zoology for their help to carry out the study.

I wish to express my deep sense of gratitude to respectable **Dr. Saroja Prabhakaran, Director, Halls of Residence,** Sri Avinashilingam Educational Trust Hostel, Coimbatore who keeps motivating us in all our ups and downs, words are insufficient to thank her for the permission, support and encouragement in completing this study.

I express thanks to my beloved parents **Mr. R. Ekambaram** and **Mrs. E. Vasanthi** and my brother **E. Nagavijay** for their motivation, encouragement and loving care.

I am glad to express my deepest gratitude to my **friends**. Words cannot express how grateful I am for their enthusiastic encouragement, motivation and timely help provided.

(Deepika E)

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

From the beginning of human civilization, plant and plant products are usually used to treat different diseases (Joshi *et al.*, 2009). As plants have substances of medicinal values, therefore, they are used to treat number of diseases since long time. Use of plants had minimal or less side effect on human beings (Doughari, 2006). With the passage of time the usage of plants is increasing in pharmaceutical industries, suggested that if a plant is used as remedy of disease then it would have some important ingredients (Nostro *et al.*, 2000).

Historically all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts, mixtures, etc. Today a substantial number of drugs are developed from plants (Fabricant and Farnsworth, 2001) which are active against a number of diseases. The majority of these involve the isolation of the active ingredients (chemical compound) found in a particular medicinal plant and its subsequent modification. In the developed countries, 25 percent of the medical drugs are based on plants and their derivatives (Principe, 2005) and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries (Gurib-Fakim, 2006).

Researchers have great interest in those substances which are derived from plants because they are versatile in their applications. Various phytochemical can be obtained from plants which are very beneficial for mankind and medicinal plants have become the richest biological resource of such chemicals which are used in manufacturing of traditional drugs as well as in modern nutraceuticals, food supplements, medicines, folk medicines, raw material and pharmaceutical intermediates for synthetic drugs (Tumwine, 2011).

The therapeutic effect of medicinal plants for the treatment of various diseases was based on the chemical compounds of these plants. The major components are organic compounds, some of which have biological activity, but none act independently and cannot replace the functions of the medicinal plant as a whole. Analysis has revealed that medicinal plants are rich in many trace elements, and it is suggested that this is an important factor in the curative effect of these plants (Olabanji *et al*., 1997; Pereira and Felcman, 1998).

Nowadays, multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (Service, 1995). In addition to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune suppression and allergic reactions (Ahmad *et al.*, 1998). This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance (Monroe and Polk, 2000), there is a constant need for new and effective therapeutic agents (Bhavnani and Ballow, 2000). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Cordell, 2000). Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world (DeBoer *et al.*, 2005).

Worldwide, infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing in developed countries, such as the United States (Pinner *et al.*, 1996)

Antimicrobial compounds are a group of chemical compounds which are synthetically or biosynthetically produced which either destroy or usefully suppress the growth and metabolism of variety of microorganisms (Lavanya & Brahmaprakash, 2011).

Herbal drugs have become increasingly popular and their use is widespread. Clear-cut proof of their efficacy in microorganisms inducing pathogenesis is yet to be explored. Various medicinal plants have been used for years in daily life to treat disease all over the world. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982) and natural products play an important role in drug development programs in the pharmaceutical industry (Baker *et al.*, 1995). It has been suggested that aqueous and ethanoic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumor and antimicrobial agents (Vlietinck *et al.*, 1995).

Pharmacologically important plants present a large source of antimicrobial agents and serve as a drug in many countries (Mahesh and Satish, 2008). This antimicrobial activity of plant is due to their constituents of oils and extracts which are used in pharmaceutical and other related fields (Hammer *et al*., 1999)

The use of plant based antimicrobials is because of lesser side effects. Natural antimicrobial compound in flora have been found to have antimicrobial activity. Additionally, the antimicrobial potential of medicinal flora may vary depending on the variety of other plants for instance fresh dried or extracted forms. Hunt for novel material with antimicrobial potential are common and medicinal flora have been considered appealing by some investigators since they are frequently used in popular medicine as remedies of many infectious

diseases. Medicinal flora has also been measured as healthy resource of life for all the people. For the extensive phase of time, herbs have been a precious store of natural goods for maintaining human wellbeing. India has prosperous folklore in use of medicinal flora to develop drugs. According to World Health Organization any plant which have material that can be used for curative purposes or which are pioneer of chemo pharmaceuticals semi synthetics new drugs is reoffered as medicinal flora (Hassanali, 2003). Medicinal flora would be the significant resource of obtaining a diversity of drugs as phytochemicals are more precise, recyclable and are supposed to have lesser side effects, phytochemicals present exceptional platform for structural variety and natural functionality which is vital for drug innovation (Verpoorte, 2002). Natural world is the basis of medicinal means and a remarkable number of contemporary drugs have been obtained from natural supply, several are based on their use in customary medicine. A variety of medicinal flora has been used for many years in day to day life to cure disease around the world (Nair *et al*., 2005). Traditionally plant accounts to be encouraging fresh drug components, as plant derived drugs have made huge aid to human fitness and well-being (Astal *et al*., 2005). Natural products from plants present novel agents for antimicrobial use. An unusual characteristic of higher flora is their ability to manufacture a huge number of organic chemicals of elevated structural variety which are also known as secondary metabolites (Naqri *et al*., 1991).

Globally, researchers are using extracts of plants for their antiviral, antibacterial, and antifungal activities. The characteristics of the plants that retard the growth of micro-organisms have been investigated in different laboratories around the world since 1926 (Bakht *et al*., 2012). Hence, in the present investigation, same efforts are continued in the progressions of searching novel therapeutics against antibiotic activity.

Pyrexia or fever is defined as an elevation of body temperature. It is a response due to tissue damage, inflammation, malignancy or graft rejection. Cytokines, interleukin, interferon and Tumor Necrosis Factor α (TNF- α) are formed in large amount under this condition, which increase PGE2 which in turn triggers hypothalamus to elevate body temperature (Rajani *et al.*, 2011). Fever is associated with symptoms of sickness behavior which consist of lethargy, depression, anorexia, sleepiness, & inability to concentrate. This increase in set point triggers increased muscle tone & shivering. However antipyretic medication can be effective at lowering the temperature which may include the affected persons comfort (Duraisankar *et al.*, 2012).

According to Ayurveda, pyrexia originates from a combination of indigestion, seasonal variations and significant alterations in daily routine (Gupta *et al.*, 2008). Due to poor hygiene practices and malnutrition, children in developing countries frequently suffer from various forms of infections which present as fevers. These fevers are often accompanied by aches and pains which all lead to morbidity and mortality (Ighodaro *et al.*, 2009).

Antipyretics are drugs which can reduce elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus which regulate the set point of body temperature. Drugs like paracetamol do not influence body temperature when elevated by factors such as exercise or increase in ambient temperature (Gomathi *et al.*, 2011).

Today a substantial number of drugs are developed from plants which are active against a number of diseases. The majority of these involve the isolation of the active ingredient (chemical compound) found in a particular medicinal plant and its subsequent modification. In the developed countries 25 percent of the medical drugs are based on plants and their derivatives and the use of medicinal

plants is well known among the indigenous people in rural areas of many developing countries (Ignacimuthu *et al*., 2009).

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 ayurveda 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 diarrhea 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 and 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, fever causing 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 use this herb for a variety of ailments like dysmenorrhoea, leucorrhoea, pre-natal and post-natal care, complicated diseases such malaria, jaundice, gonorrhea and general.

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 anti-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), hepato protective (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 neo andrographolide (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).

Considering all the above information, the present investigation has a broader objective of comprehensive evaluation of *in vivo* antipyretic activity using the leaf of *Andrographis paniculata* in different formulations such as capsule and leaf powder. The *in vitro* studies were done to show the antimicrobial efficacy of *Andrographis paniculata*.

Thus, the specific objectives of the present study are

To

- Carry out *in vivo* studies on the antipyretic activity of ethanol extract and capsule of *Andrographis paniculata* leaves.
- Evaluate the antibacterial efficacy of ethanol extract and capsule of *Andrographis paniculata* leaves.
- Evaluate the antifungal efficacy of ethanol extract and capsule of *Andrographis paniculata* leaves

2. REVIEW OF LITERATURE

The review of literature pertaining to the topic "**Evaluation of Antipyretic and Antimicrobial efficacy of *Andrographis paniculata* in different formulations"** is presented in the following headings:

2.1 Natural plant product research

Since prehistoric times, the treatment and cure of diseases has been one of the primary concerns of mankind. Local practitioners have used indigenous plants and herbs for centuries all over the world to treat a variety of ailments and these have exhibited clear pharmacological activities. The history of medicine is an account of mankind's efforts to deal with human illness and diseases ranging from primitive attempts of preliterate man to the present complex array of therapeutic specialties (Buchman and Germerey, 1980).

The earliest written records on medicinal applications of plants date back to 2600 BC and mentioned the existence of a sophisticated medicinal system in Mesopotamia, compromising about 1000 plant – derived medicines. Egyptian medicine dates back to about 2900 BC, but its most useful preserved record is the "Ebers Papyrus" from about 1550 BC, containing more than 700 drugs, mainly of plant origin (Borchardt 2002; Cragg and Newman, 2013; Sneader, 2005). Traditional Chinese Medicine (TCM) has been extensively documented over thousands of years (Unschuld, 1986), and the documentation of the Indian Ayurveda system dates back to the 1st millennium BC (Patwardhan, 2005).

The knowledge on the medicinal applications of plants in the western world is mainly based on the Greek and Roman culture. Among which, the compendia written by the Greek physician Dioscorides (1st century AD) and by the Romans Pliny the Elder (1st century AD) and Galen (2nd century AD) (Sneader, 2005) are of great significance. The Arabs preserved a large amount of the Greco-Roman knowledge during the Dark and Middle ages (5th to 12thcenturies), and

complemented it with their own medicinal expertise, and with herbs from Chinese and Indian traditional medicines (Cragg and Newman, 2013).

The invention of printing press by Johannes Gutenberg led to a resurrection of the Greco-Roman herbal knowledge in the 15th and 16th century. This led to the compilation of several herbal books that were widely distributed in Europe: The Mainz Herbal (Herbarius Monguntinus, 1484) and The German Herbal (1485), both edited by Gutenberg's partner Peter Schoffer, Herbarium Vivae Eicones by Hieronymus Bock (1546) that was written in German, and De Historia Stripium by Leonart Fuchs that was published in Latin in 1542 and also in German in the following year (Sneader, 2005).

During this period, medicinal plants were only applied on an empirical basis, without mechanistic knowledge on their pharmacological activities or active constituents. Rational drug discovery from plants started at the beginning of the 19th century, when the German apothecary assistant Friedrich Serturner succeeded in isolating the analgesic and sleep inducing agent from opium.

This trend resulted in the examination of other medicinal herbs, and during the following decades of the 19th century, many bioactive natural products, primarily alkaloids (e.g., quinine, caffeine, nicotine, codeine, atropine, colchicines, cocaine and capsaicin) could be isolated from their natural sources (Corson and Crews, 2007; Felter and Lloyd, 1898; Hosztafi, 1997; Kaiser, 2008; Kruse, 2007 and Sneader, 2005). These efforts were then followed to produce natural products by chemical synthesis aimed at production at higher quality and lower costs. Salicylic acid was the first natural compound produced by chemical synthesis in1853 (Kaiser, 2008).

After the discovery of penicillin (1928), an era of drug discovery from microbial sources was initiated in the 1930's. This paved way to the scientific and financial foundation of the modern pharmaceutical industry after World War II. Despite the advent of combinatorial chemistry and High Throughput Screening (HTS) campaigns during the last decades, the impact of natural products for drug

discovery is still very high. Of the 1073 new chemical entities belonging to the group of small molecules that had been approved between1981 and 2010, only 36% were purely synthetic, while more than the half were derived or inspired from nature (Newman and Cragg, 2012). A substantial number of these compounds have been discovered in higher plants, anti-cancer agents, e.g., paclitaxel and its derivatives from yew (Taxus) species, vincristine and vinblastine from Madagascar periwinkle (*Catharanthusroseus* (L.) G. Don), and campothecin and its analogs initially discovered in the Chinese tree *Campot hecaacuminat* Decne (Cragg and Newman, 2013 and Kinghorn *et al*., 2011) and the important antimalarial and potential anti-cancer agent artemisinin originally derived from the traditional Chinese herb *Artemisia annual.*

2.2 Traditional medicine

Traditional Medicine (TM) holds great potential to improve people's health and wellness. It is an important, yet often underestimated part of health care. TM is found in almost every country in the world and the demand for its services is increasing every day. TM can contribute to addressing a number of global health challenges of the 21st century, in particular in the area of chronic, non-communicable diseases and population aging. Traditional medical knowledge is experiencing increased attention worldwide in light of global health care demand and the significant role of traditional medicine in meeting the public health needs of developing countries.

TM describes a group of health care practices and products with a long history of use. It frequently refers to medical knowledge developed by indigenous cultures that incorporate plant, animal and mineral-based medicines, spiritual therapies and manual techniques designed to treat illness or maintain wellbeing (WHO, 2003).

TM tends to be practiced outside of allopathic medicine, which is the dominant system of medicine in the developed world. In many cultures, TM functions as a comprehensive system of health care refined over hundreds or

even thousands of years. Some of the best-known TM systems include traditional Indian (Ayurveda) medicine, traditional Chinese medicine (TCM), and traditional Arabic (Unani) medicine.

Ayurveda is a medical system primarily practiced in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment (Morgan, 2002). The term comes from the Sanskrit root Au (life) and *Veda* (knowledge). As the name implies it is not only the science of treatment of the ill but covers the whole gamut of happy human life involving the physical, metaphysical and the spiritual aspects. Ayurveda is gaining prominence as the natural system of health care all over the world. Today this system of medicine is being practiced in countries like Nepal, Bhutan, Sri Lanka, Bangladesh and Pakistan, while the traditional system of medicine in the other countries like Tibet, Mongolia and Thailand appear to be derived from Ayurveda.

TCM has been used by Chinese people from ancient times. Although animal and mineral materials have been used, the primary source of remedies is botanical. Of the more than 12,000 items used by traditional healers, about 500 are in common use. Botanical products are used only after some kind of processing, which may include, for example, stir-frying or soaking in vinegar or wine. In clinical practice, traditional diagnosis may be followed by the prescription of a complex and often individualized remedy. TCM is still in common use in China. More than half the population regularly uses traditional remedies, with the highest prevalence of use in rural areas. About 5000 traditional remedies are available in China; they account for approximately one fifth of the entire Chinese pharmaceutical market (Li, 2000).

The modern use of Arab botanical medicines has historical roots in ancient Arabic medicine. Arab herbalists, pharmacologists, chemists and physicians in the middle ages adopted the ancient medicinal practices of Mesopotamia, Greece, Rome, Persia and India. Medical innovations introduced by Arab

physicians included: the discovery of the immune system, the introduction of microbiological science and the separation of medicine from pharmacological science.

The widespread use of TM has resulted in traditional health care becoming a lucrative, multinational business. Billions of U.S. dollars are spent annually on traditional medicine in many developed countries. For example, in 2012, 32 billion dollars was spent in the United States of America on dietary supplements, an amount projected to increase to 60 billion dollars in 2021. In developing countries, more money may be spent on TM than on allopathic care. New drug development is an expensive and risky venture. Pharmaceutical companies invest billions of dollars annually in the hope of developing new chemical entities that are safe and effective, and that can be manufactured in a cost effective way. It is estimated that for every 10,000 pure compounds that are biologically evaluated, only one achieves regulatory approval. A single approval can take upwards of a decade and cost hundreds of millions of dollars (DiMasi, 2003).

Traditional medicine is commercialized and exported in a variety of settings. Some TM holders have chosen to market their knowledge outside of traditional settings. China, for example, promotes global TCM use to foster domestic economic development. Exports of TCM products from China generate billions of U.S. dollars in revenue annually. China's situation is not unique. In 2004, China accounted for only five percent of the global market for TM (WHO, 2005).

2.3 An overview of *Andrographis paniculata* (Burm.f) Nees

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 (Kanokwan and Nobuo, 2008). 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).

2.3.1 Classification:

Kingdom : Plantae
Sub-kingdom : Tracheobionta
Division : Angiosperma
Class : Dicotyledonae
Sub-class : Gamopetalae
Order : Personales
Family : Acanthaceae
Genus : *Andrographis*
Species : *paniculta*

2.3.2 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.

2.3.3 Habitat

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)

2.3.4 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 5 particle, 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 glandular hairy. 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 and Mishra *et al*., 2007)

2.4 Therapeutic efficacy of *Andrographis paniculata*

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 and Oktay, *et al*., 2003). Several studies have been reported the antioxidant activities of

A. paniculata and its constituents. Verma and Vinayak 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 RO Sand 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).

Andrographolide has been reported to significantly reduce the inflammation caused by histamine, dimethyl benzene, and adrenaline (Deng, *et al.*, 1978). Overproduction of NO and prostaglandin E2 (PGE2), because of the expression of inducible isoforms of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), plays a significant role in the inflammatory processes of activated macrophages. The secretion of pro inflammatory cytokines from macrophages stimulated and promoted by lipopolysaccharide, which causes induction of iNOS, results in increased production of NO. The methanol extract of *A. paniculata* and andrographolide incubated with macrophages have been reported to inhibit LPS-stimulated NO production in a concentration-dependent manner (Batkhuu, *et al.*, 2002 and Liu *et al.*, 2007). Chiou *et al.* observed that andrographolide inhibits lipopolysaccharide induced nitric oxide (NO) production and inducible NO synthase (iNOS) expression in the murine macrophage like cell line RAW 264.7. Administering andrographolide to rats fully restored the maximal contractile response of the thoracic aorta to phenylephrine after incubation with LPS and alleviated the decrease in the mean arterial blood pressure of

anesthetized rats. Andrographolide has also been reported to suppress IL-2 production and T-cellproliferation in a mixed lymphocyte reaction and to inhibit dendritic cell maturation and antigen presentation (Iruretagoyena *et al*., 2006.].

Natural products are recognized as sources for drugs used to treat several human ailments including cancers. Vincristine, irinotecan, etoposide, and paclitaxel are examples of many natural pharmaceuticals derived from plants (Da Rocha *et al*., 2001). Despite the discovery of numerous drugs of natural origin, searching for new anticancer agents is still necessary to provide drugs that are less toxic and more effective and to increase their variety and availability. Samples with pharmacological usage should be accounted for when selecting plants to treat cancer because several ailments reflect disease states bearing relevance to cancer or cancer like symptoms (Cordell *et al.,* 1991). Andrographolide exhibited potentcy toxic activity against KB (human epidermoid leukemia) and P388 (lymphocytic leukemia) cells (Siripong *et al.,* 1992). Among the diterpenoid lactones isolated from the ethyl acetate fraction of *A. paniculata*, andrographolide had strong anticancer activity by inducing cell differentiation in mouse myeloid leukemia cells (Matsuda, *et al*., 1994). Andrographolide was found to inhibit the proliferation of various cell lines including leukemia, breast cancer, lung cancer, and melanoma cells (Nanduri *et al.,* 2004 and Rajagopal *et al.,* 2003). Furthermore, this compound has strong anticancer activity against human colorectal carcinoma LoVo cells by inhibiting cell cycle progression (Shi *et al*., 2008). A potent growth inhibitory effect of andrographolide has been demonstrated in acute promyelocytic leukemia cells (HL60andNB4) that are mediated by inducing cell differentiation and apoptosis (Li *et al*., 2007 and Manikam and Stanslas, 2009). Andrographolide was also reported to suppress the adhesion of gastric cancer cells which express high-level sialyl Lewis X to human vascular endothelial cells by blocking E-selectin expression and, thus, may represent a candidate therapeutic agent for cancer (Jiang *et al*., 2007). Lim *et al*., 1990, demonstrated that the anticancer mechanisms for andrographolide

include the inhibition of Janus tyrosine kinases-signal transducers and activators of transcription, phosphatidylinositol 3-kinase and NF-κB signaling pathways, suppression of heat shock protein 90, cyclins and cyclin-dependent kinases, metalloproteinase and growth actors, and the induction of tumor suppressor proteins p53 and p21, leading to the inhibition of cancer cell proliferation, survival, metastasis, and angiogenesis.

In vivo models of the anticancer activity of andrographolide have been used against MCF-7 and HT-29 tumor xenografts and B16F0 melanoma (Li, *et al.,* 2007). In a radiation therapy study, andrographolide was found to sensitize Ras transformed cells and significantly delay tumor growth (Hung, *et al*., 2010). Sheeja and Kuttan, 2007 demonstrated that *A. paniculata* extract or andrographolide alone could stimulate cytotoxic T lymphocyte production through the enhanced secretion IL-2 and IFN-c by T cells, thereby inhibiting tumor growth in vivo. Inhibition of angiogenesis is currently perceived as a promising strategy in treating cancer. In a significant invention, *A.paniculata* and andrographolide alone were found to inhibit tumor-specific angiogenesis by regulating the production of various pro-and anti angiogenic factors, such as pro inflammatory cytokines, NO, vascular endothelial growth factor, IL-2, and the tissue inhibitor of metalloproteinase1 (Sheeja and Kuttan, 2007). A recent study demonstrated that andrographolide inhibits breast cancer cell proliferation, migration, and cell cycle arrest at the G2/M phase and induces apoptosis through acaspase–independent pathway. Their experimental evidence suggests that andrographolide attenuates endothelial cell motility and tumor-endothelial cell interaction (Kumar *et al.,* 2012). The antitumor activity of andrographolide in an in vivo model was correlated with the down regulation of PI3 kinase/Akt activation, inhibition of proangiogenic molecules, such as OPN, and VEGF expressions (Kumar *et al*., 2012).

Purified andrographolide (1mg/kg body weight) or intragastric administration of ethanol extracts of the stems and leaves (25mg/kg body weight)

to mice stimulate antibody production and the delayed-type hypersensitivity response to sheep red blood cells (Puri *et al.*, 1993). The extract and purified andrographolide were also reported to stimulate an innate immune response in mice, which was measured according to the macrophage migration index, phagocytosis of leucine-labelled Escherichia coli, and proliferation of splenic lymphocytes stimulated by *A.paniculata* extract (Puri *et al.,* 1993).The immunomodulatory property of a diterpene lactone andrographolide was reported to be associated with the enhancement of the proliferation of human peripheral blood lymphocytes, as well as the production of key cytokines and the expression of Y Xu 21 immune activation markers in whole blood cells in culture in vitro (Panossian *et al.*,1999). *In vivo* immune responses, such as an antibody response to a thymus-dependent antigen and delayed-type hypersensitivity, were considerably lessened in mice treated with andrographolide. In addition, Iruretagoyena *et al*. 2005 reported that andrographolide enhanced the tolerogenic properties of immature dendritic cells (DCs) in experimental autoimmune encephalomyelitis (EAE) by inhibiting NF-kappa B activation in murine DCs. Andrographolide was also reported toreduce IFN-$\gamma$and IL2 production in murine T-cells stimulated with concanavalin A (Con A) *in vitro* (Burgos *et al.*, 1997). Moreover, andrographolide was reported to inhibit the production of TNF-α and IL-12 in macrophages stimulated by lipopolysaccharide (Qin *et al.,* 2006).

Liver diseases of various origins remain a serious health problem and a major cause of mortality. In the absence of reliable hepatoprotective drugs in modern medicine, herbs and plants play a vital role in managing several liver disorders (Maiti *et al.,* 2006 and Mukherjee *et al.,* 2002). Extensive literature related to the hepatoprotective activity of molecules from herbal sources shows that there is a vast array of molecules exhibiting potent hepatoprotective efficacy. The Indian systems of medicine have long used *A. paniculata* as a

hepatostimulant and hepatoprotective agent (N. P. Trivedi and U. M. Rawal, 2001).

A recent study showed that andrographolide attenuated concanavalin A-induced liver injury and inhibited hepatocyte apoptosis (Shi *et al.*, 2012). The effect of andrographolide was found to be more potent than silymarin against acetaminophen-induced reduction of the volume and contents of bile. Andrographolide was also shown to protect against ethanol-induced hepatotoxicity in mice with an equivalent efficacy of silymarin (Singha *et al.*, 2007). Oral pre- and post-treatments of adult rats with an extract of *A. paniculata* were protective against ethanol-induced increase in serum transaminases. A protective effect of a single oral dose each of the extract and of andrographolide has been studied in carbon tetrachloride (CCl4) induced hepatic microsomal lipid per oxidation. Rana *et al*. 1991 reported the hepatoprotective effects of the crude alcohol extract of leaves against CCl4-induced liver damage; these effects have had also been established against paracetamol induced toxicity in an *ex vivo* rat model of isolated hepatocytes (Visen *et al*., 1993). Plant extracts of *A.paniculata* showed hepatoprotective characters consistent with the folk use and pharmacology (Kunwar, *et al*., 2010).

Antimicrobial drugs have caused a dramatic change not only in the treatment of infectious diseases but to the fate of mankind. Antimicrobial chemotherapy has made noteworthy advances, resulting in positive observations that infectious diseases might be dominated in the near future. However, in reality, emerging and reemerging infectious diseases have indicated a countercharge from infections. Infections with drug-resistant organisms hang back an imperative problem in clinical practice that is complicated to explain. If an unsuitable antimicrobial agent is preferred over the treatment of infection with drug-resistant microorganisms, the therapy may not achieve beneficial effects and may lead to a worse prognosis. *A. paniculata* and andrographolide have been reported to exhibit potent antimicrobial activity against various microbial

organisms. In vitro antibacterial activity of the crude powder of *A. paniculata* has been reported against *Salmonella, Shigella, E. coli, and streptococci,* and *Staphylococcus aureus*, even at a concentration of 25mg/ml. Singha *et al.*, 2003 found significant antibacterial activity in an aqueous extract with andrographolide. A similar result was found in a crude aqueous extract of leaves that exhibit significant antimicrobial activity against gram-positive *S. aureus, methicillin resistant S. aureus,* and gram-negative *Pseudomonas aeruginosa* (Zaidan *et al.,* 2005). Significant activity against entero hemorrhagic strains of *E. coli* was found in the ethanol extract of *A. paniculata* (Voravuthikunchai and Limsuwan, 2006). The virucidal activity of andrographolide has been reported against herpes simplex virus 1 (HSV1) without having any significant cytotoxicity (Wiart *et al.*, 2005). At a concentration of 0.05mg/mL of a chloroform extract of *A. paniculata*, the plant completely inhibits malarial parasitic growth within 24h of incubation; and the same inhibition has been noted within 48h with methanol extract concentration of 2.5mg/mL (Rahman *et al.*, 1999). A methanol extract was found to inhibit *Plasmodium falciparum* substantially at a 50% inhibitory concentration (IC50) of 7.2μg/ml (Mishra *et al.*, 2009).The ethanol extract of *A.paniculata* was effective against upper respiratory tract infection (Poolsup *et al.*, 2004). The antimicrobial activity of *A. paniculata* against nine bacterial strains, *Salmonella typhimurium, E. coli, Shigella sonnei, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumonia, Streptococcuspyogenes, Legionella pneumophila,* and *Bordetella pertussis,* has also been reported (Xu, *et al.*, 2006).

The antiviral activities of plant extracts have been renewed and have been the topic of passionate scientific investigation. Several medicinal plant extracts have shown antiviral activities against some RNA and DNA viruses. Among these plants is *A. paniculata* which exhibits a neutralizing activity against the human immunodeficiency virus (HIV) (Chang *et al.,* 1991). Andrographolide was investigated for antiviral activity against herpes simplex virus (HSV) (Wiart *et al.,* 2005 and Seubsasana *et al.*, 2011). Lin *et al.*, 2008 demonstrated that 25μg/ml

of ethanol extract of *A. paniculata* and 5μg/mL of andrographolide effectively inhibit the expression of Epstein-Barrvirus (EBV) lyticproteins, Rta, Zta, and EA-D, during the virally ticcyclein P3HR1 cells. A recent study has demonstrated that *A.paniculata* has the most evidence-based complementary and alternative medicine 5 antiviral inhibitory effects among six medicinal plants tested against DENV1-infected VeroE6 cells (Tang *et al.*, 2012).

2.5 Antipyretic potency of *Andrographis paniculata*

In Asian countries, *A. paniculata* has been widely used for its antipyretic, analgesic, protozoacidal, antihepatotoxic, anti-HIV, immune stimulant, anticancer effects (Nanduri *et al.*, 2004). It had been reported that andrographolide, with oral doses of 100 and 300mg/kg, produced a significant antipyretic effect after 3h administration of brewer's yeast-induced fever in rats (Madav *et al.*, 1995.]. In addition, doses of 180 or 360mg/kg of andrographolide were also found to relieve fever in humans by the third day after administration (Thamlikitkul *et al.,* 1991). The different routes of administration between these experiments could contribute to this discrepancy (Madav *et al.*, 1995).

Suebsasana *et al.,* 2009 attributed that significant effect of *Andrographis paniculata* is due to the presence of Andrographolide (1) and 14-deoxy-11, 12-didehydro andrographolide (2). *A. paniculata* extracts are reported to have antiviral, antipyretic, immune stimulant and anticancer activities. In this study, 1 and its 14-acetyl- (4) and 3, 19 isopropylidenyl- (3) derivatives, as well as 2 and its 3, 19-dipalmitoyl-derivative (5), were intraperitoneally tested for their antipyretic and acute toxicity effects in animal models. The results showed that, /in a baker's yeast-induced fever model, 3 and 5 significantly reduced rats' rectal temperature ($p < 0.05$). From this study, 3 and 5 are the most interesting derivatives, showing much greater potency than their parent compounds. These could be further developed as antipyretic agents, without any serious toxicity.

According to Akintola *et al.,* 2018, the antipyretics in common use include acetaminophen, aspirin and other non-steroidal anti-inflammatory drugs

(NSAIDS). Most of these antipyretic drugs inhibit the enzyme cyclo oxygenase 2 (COX-2) with high selectivity but are toxic to the hepatic cells, glomeruli cortex of the brain and heart muscles whereas natural COX-2 inhibitors have lower selectivity with fewer side effects. A natural antipyretic agent with reduced or no toxicity is therefore essential.

Intragastric administration of an ethanol extract of the aerial parts (500mg/kg body weight) to rats decreased yeast-induced pyrexia (Deng 1982). The extract was reported to be as effective as 200 mg/kg body weight of aspirin, and no toxicity was observed at doses up to 600 mg/kg body weight (Gupta 1993). Intragastric administration of andrographolide (100 mg/kg body weight) to mice decreased brewer's yeast-induced pyrexia (Gupta 1990). Intragastric administration of deoxy andrographolide, andrographolide, neoandrographolide or 11, 12-didehydro- 14-deoxyandrographolide (100 mg/kg body weight) to mice, rats or rabbits reduced pyrexia induced by 2, 4-dinitrophenol or endotoxins (Chiou *et al.,* 1998)

3. MATERIALS AND METHODS

The materials used and methods adopted in the present study entitled **"Evaluation of Antipyretic and Antimicrobial efficacy of *Andrographis paniculata* in different formulations"** is furnished below.

3.1 COLLECTION OF PLANT MATERIALS

The leaf powder of *Andrographis paniculata* (Fig.1) and the capsule which contains drug of the same leaves (Fig.2) was donated by Siddha Regional Research Institute, Poojapura, Tiruvananthapuram

Fig.1 *Andrographis paniculata* leaf powder

Fig.2. *Andrographis paniculata* Capsules

3.2 PREPARATION OF THE EXTRACT

Ten grams of leaf powder of *Andrographis paniculata* was mixed with 100ml of Hydro ethanol. 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.

Simultaneously the drug inside the capsule about ten grams is separated out and dissolved in 100ml of distilled water. This was then further used for the experiments.

3.3 ANTIMICROBIAL ASSAY

The experimental procedure to analyze the antimicrobial efficacy of *Andrographis paniculata* is presented below. Antibacterial and antifungal activity studies were carried out by agar diffusion method (Barry *et al*., 1976)

3.3.1 Test microorganisms

The seven bacterial strains and the six fungal strains used in the present study were the clinical isolates obtained from P.S.G. Hospitals, Coimbatore. The bacteria used were *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus, Pseudomonas aeruginosa, Shigella flexneri and Proteus vulgaris.* The fungal strains used were *Aspergillus niger* and *Aspergillus flavus.* The pure cultures of different pathogens were grown overnight in sterile nutrient broth and incubated at 37°C for 24 hours.

3.3.2 Antibacterial activity

3.3.2.1 Principle

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a

confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

3.3.2.2 Reagents

1. Muller Hinton Agar Medium

The medium was prepared by dissolving 5.7 g of the commercially available Muller Hinton Agar Medium (Hi Media) in 150ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Standard drug Ciprofloxacin

Ciprofloxacin was dissolved in sterile water for injection (10 µg/ml) and used

3.3.2.3 Procedure

Petriplates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacterial strains. Wells were cut and 20 µl of the plant extract and drug were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the inhibition zone formed around the well.

3.3.3 ANTIFUNGAL ACTIVITY

3.3.3.1 Reagents

1. Rose Bengal Chloramphenicol Agar medium

The commercially available (HiMedia) Rose Bengal Chloramphenicol agar medium (1.6 g) was suspended in 50ml of distilled water. The medium was dissolved completely by boiling and was then autoclaved at 15 lbs pressure (121ºC) for 15 minutes.

2. Standard drug Fluconazole

Fluconazole was dissolved in sterile water for injection (30µg/ml) and used.

3.3.3.2 Procedure

Petriplates containing 20ml Rose Bengal Chloramphenicol medium were seeded with 24hr culture of bacterial strains. Wells were cut and 20 µl of the plant extract and drug were added. The plates were then incubated at 37°C for 24 hours. The antifungal activity was assayed by measuring the inhibition zone formed around the well.

3.4. ANTIPYRETIC ACTIVITY

3.4.1 Pharmacological screening

Experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC-Resolution No. 13, 31-7-2010) and carried out in accordance with CPCSEA guidelines. The certificate of ethical clearance is enclosed as Annexure-I

3.4.2 Experimental animals

Healthy Swiss albino mice, *Mus musculus* (20±5 gm) were used for the study. The animals were obtained from Kerala Veterinary Animal Sciences University, Mannuthy, Thrissur, Kerala. Animals were kept in polypropylene cages with sawdust bedding and maintained in laboratory conditions. Standard pellets were given as diet and water was provided ad libitum. The animals were acclimatized to laboratory condition for about one week before commencement of the experiment. The experiments were performed after the approval from the institutional Animal Ethical Committee and in accordance with the recommendation for the proper care and use of the laboratory animals

3.4.3 Treatment procedure

The method of yeast induced pyrexia was used. Healthy Swiss albino mice which have not been used for any previous experiments were divided into suitable groups with four in each (Fig.3). Prior to induction of pyrexia, initial rectal temperatures were recorded utilizing digital thermometers. These thermometers

were inserted into the rectum. Group I serve as control which receive saline (5ml/kg). Group II serve as positive control which receive paracetamol (150 mg/kg). Group III and IV serve as varying doses (200mg/kg and 400 mg/kg) of ethanol extract of Andrographis paniculata. Group V and VI serve as varying doses (200mg/kg and 400 mg/kg) of Andrographis paniculata capsules. The designation of the animal groups and treatment details were as follows:

Group I → Saline control

Group II → Standard Paracetamol

Group III → EEAP (200mg/kg)

Group IV → EEAP (400 mg/kg)

Group V → AP Capsules (200 mg/kg)

Group VI → AP Capsules (400mg/kg)

EEAP- Ethanol extract of *Andrographis paniculata*

The mice except the negative control will be subcutaneously injected with 10ml/kg of 20% aqueous suspension of *Saccharomyces cerevisiae*. Temperature will be recorded at 1hr intervals. Animals were then rehabilitated after completion.

Fig.3 Experimental set up

3.5 STATISTICAL ANALYSIS

All the data expressed as mean ±SD were evaluated by one-way analysis of variance (ANOVA), followed by DMRT for multiple comparisons and F values of $P < 0.05$ were considered as statistically significant.

4. RESULTS

The results pertaining to the study "**Evaluation of Antipyretic and Antimicrobial efficacy of *Andrographis paniculata* in different formulations**" are presented in the following headings:

4.1 Anti-microbial activity

4.1.1 Anti-bacterial activity

4.1.2 Anti-fungal activity

4.2 Anti-pyretic activity

4.1 ANTIMICROBIAL ACTIVITY

Antimicrobial resistant bacteria are the causes of numerous clinical problems worldwide. Infectious disease caused by resistant microorganisms is responsible for increased health costs as well as high morbidity and mortality, particularly in developing countries. The increase in the prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs and has necessitated the search for new antimicrobials from natural sources.

4.1.1 Anti-bacterial activity

The leaves extract were evaluated for its antibacterial activity against seven clinical bacterial isolates gram positive bacteria include *Klebseilla pneumonia, Proteus vulgaris, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* Gram negative bacteria include *Streptococcus and Shigella flexneri.* Table I describes the antibacterial activity of ethanol, ethanol extracts and capsule of the *A. paniculata* against selected bacterial isolates. Plate I shows the zone of inhibition of *A. paniculata* against bacterial isolates.

From the Table I it was observed that the zone of inhibition was found to be maximum in the *A.Paniculata* capsule and was found to be more active against *Klebseilla pneumonia* (22mm), *Proteus vulgaris* (21mm), *Staphylococcus aureus* (17mm), *Escherichia coli* (14mm), *Pseudomonas aeruginosa* (21mm), *Streptococcus* (13mm) and *Shigella flexneri* (*21*mm). The ethanol extracts and was found to be more active against *Klebseilla pneumonia* (11mm), *Proteus vulgaris* (17mm), *Staphylococcus aureus* (16mm), *Escherichia coli* (15mm) *Pseudomonas aeruginosa* (22mm), *Streptococcus (*16mm) and *Shigella flexneri* (*20*mm). The positive control Ciproflaxin exhibited the zone of inhibition of 23mm, 22mm, 24mm, 23mm, 24mm, 24mm, and 22mm against *Escherichia coli, Pseudomonas aeruginosa, Klebseilla pneumonia, Proteus vulgaris, Staphylococcus aureus, Streptococcus and Shigella flexneri.*

TABLE I: Antibacterial activity of the ethanol extract and capsules of *Andrographis paniculata* against the selected bacterial isolates

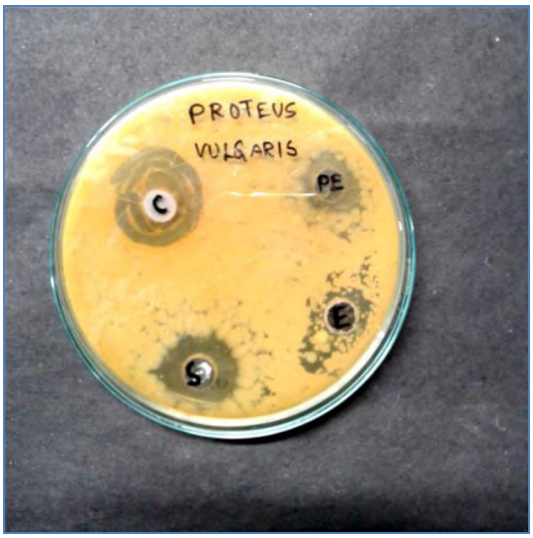
Bacterial isolates	Zone of inhibition (mm)		
	Control (Ciproflaxin)	EEAP	APC
Escherichia coli	23±1.52	15±1.53	14±1.1
Pseudomonas aeruginosa	22±1.02	22±1.02	21±1
Klebseilla pneumonia	24±2.01	11±1.52	22±1.2
Proteus vulgaris	23±1.52	17±3.7	21±1
Staphylococcus aureus	24±2.01	16±2.64	17±3.7
Streptococcus	24±2.01	15±1.53	13±2.08
Shigella flexneri	22±1.2	20±1	21±1

- **Values are expressed as mean±SD of the triplicates**

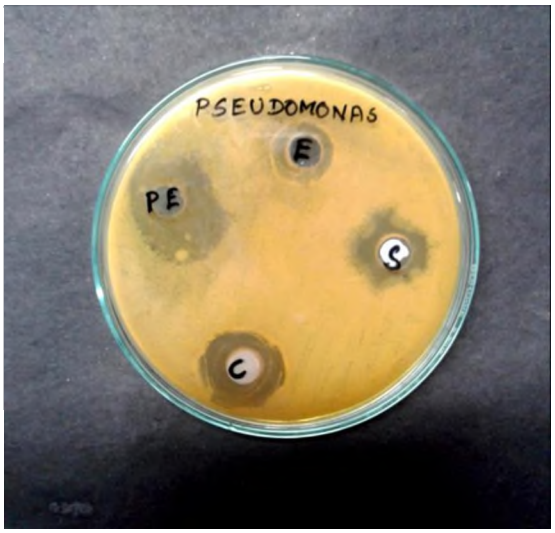
EEAP-Ethanol Extract of *Andrographis paniculata*

APC- *Andrographis paniculata* Capsules

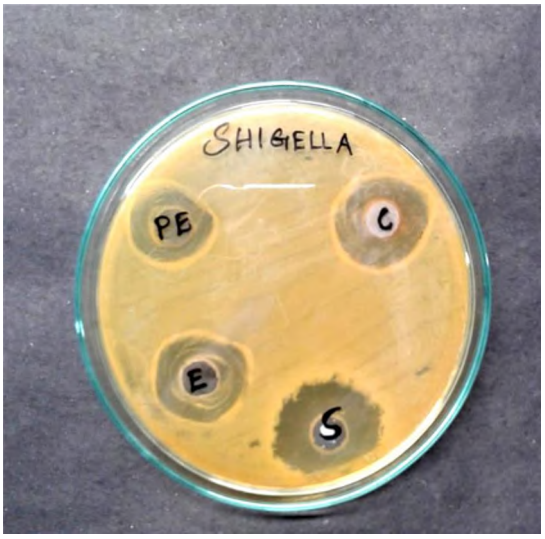
Staphylococcus aureus



Proteus vulgaris



Pseudomonas aeruginosa



Shigella flexneri

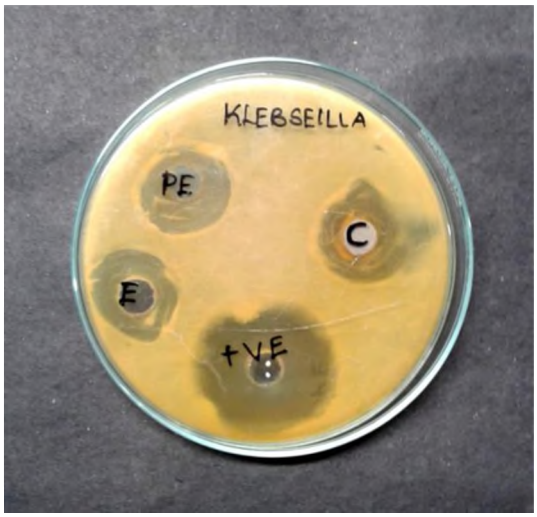
PE~EEAP- Ethanol Extract of *Andrographis paniculata*

C~APC- *Andrographis paniculata* Capsules

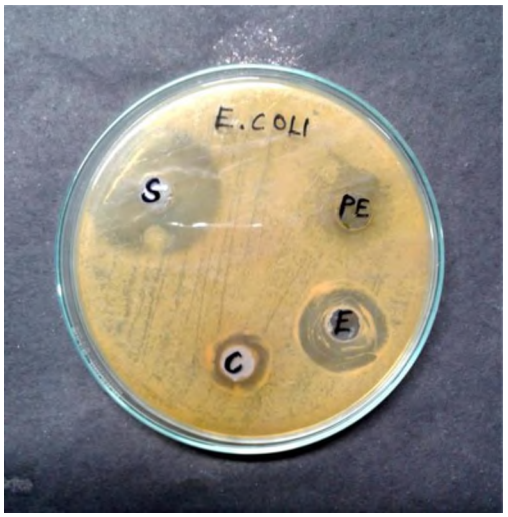
S- Standard (Ciprofloxin)

E-Ethanol-Vehicle control

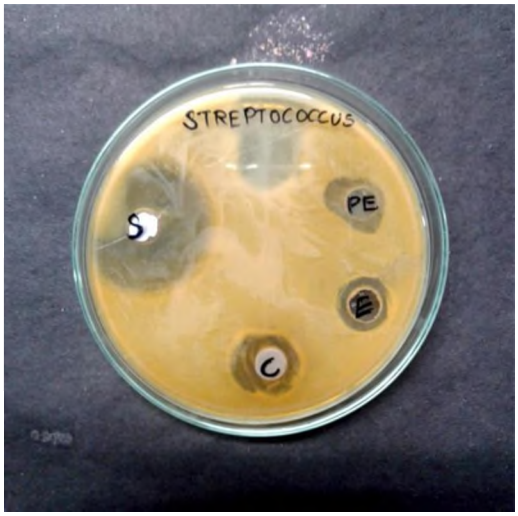
E- Ethanol C- Capsule



Klebseilla pneumonia



Escherichia coli



Streptococcus

PE~EEAP- Ethanol Extract of *Andrographis paniculata*

C~APC- *Andrographis paniculata* Capsules

S- Standard (Ciprofloxin) E-Ethanol-Vehicle control

E- Ethanol C- Capsule

Fig.4 Antibacterial activity of the ethanol extract and capsules of *Andrographis paniculata* against the selected bacterial isolates

4.1.2 Antifungal activity

The antifungal activity was determined against the fungal isolates namely *Aspergillus niger,* and *Aspergillus flavus*. Table.II depicts the antifungal activity of *Andrographis paniculata* leaf extracts. The capsule of *Andrographis paniculata* showed the maximum activity against *Aspergillus flavus* (18mm) and *Aspergillus niger* (15mm) in comparison with ethanol extract. The ethanol extract exhibited the zone of inhibition of 16mm and 15mm against *Aspergillus flavus* and *Aspergillus niger*. The inhibition zone for the tested fungi ranged from 10-19mm indicating a remarkable antifungal effect when compared with that of Fluconazole, the positive control, which ranged from 21 - 25mm. Fig.5 shows the zone of inhibition of *Andrographis paniculata* against the fungal isolates.

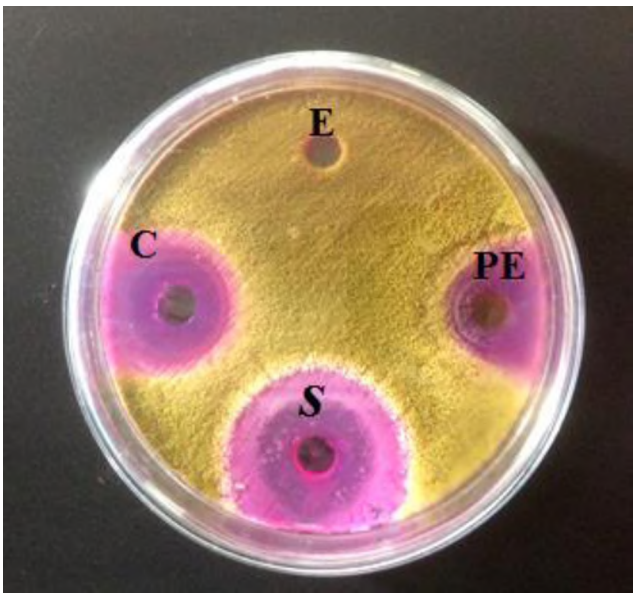
TABLE II Antifungal activity of the ethanol extract and capsules of *Andrographis paniculata* against the selected fungal isolates

Fungal isolates	Zone of inhibition in diameter (mm)		
	Control	EEAP	APC
Aspergillus flavus	20±1.50	16±2.64	18±1.52
Aspergillus niger	22±1.53	15±.53	15±1.53

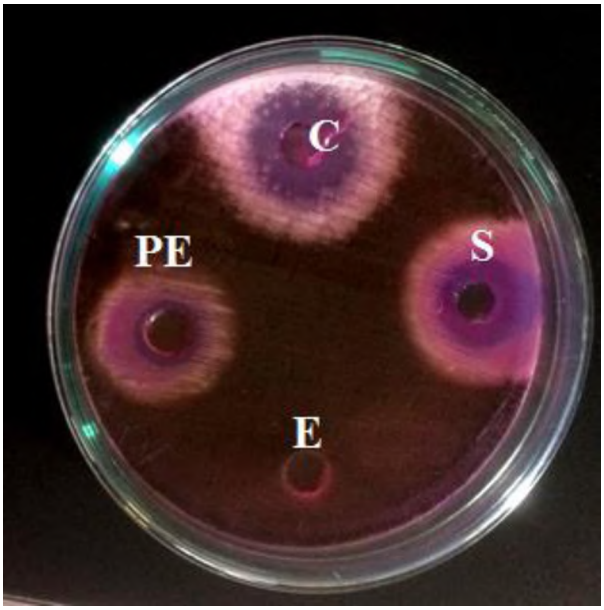
- **Values are expressed as mean±SD of the triplicates**

EEAP-Ethanol Extract of *Andrographis paniculata*

APC- *Andrographis paniculata* Capsules



Aspergillus flavus



Aspergillus niger

Fig.5: Antifungal activity of the ethanol extract and capsules of *Andrographis paniculata* against the selected fungal isolates

4.2 ANTIPYRETIC ACTIVITY

The efficacy of the *Andrographis paniculata* extract 400mg/kg was not significant at 1h but it showed sigificant activity at 3h and 5h P<0.005. The paniculata extract 200mg/kg showed more significant (P<0.05) antipyretic activity at 1h and 3h compared to control group than standard drug paracetamol. The efficacy of the *Andrographis paniculata* capsule 200mg/kg was more consistent and significant P<0.05 than *Andrographis paniculata* extract (400mg/kg) were as the doses (200 and 400mg/kg) of *Andrographis paniculata* capsule showed more significant at 1h, 3h and 5h. The higher level of inhibition of pyrexia with *Andrographis paniculata* capsule (200 and 400 mg/kg) administration of EEAP, capsule at 200 mg/kg and paracetamol (150 mg/kg) revealed the greater efficacy than *Andrographis paniculata* extract and paracetamol was observed (Table III and Fig.6).

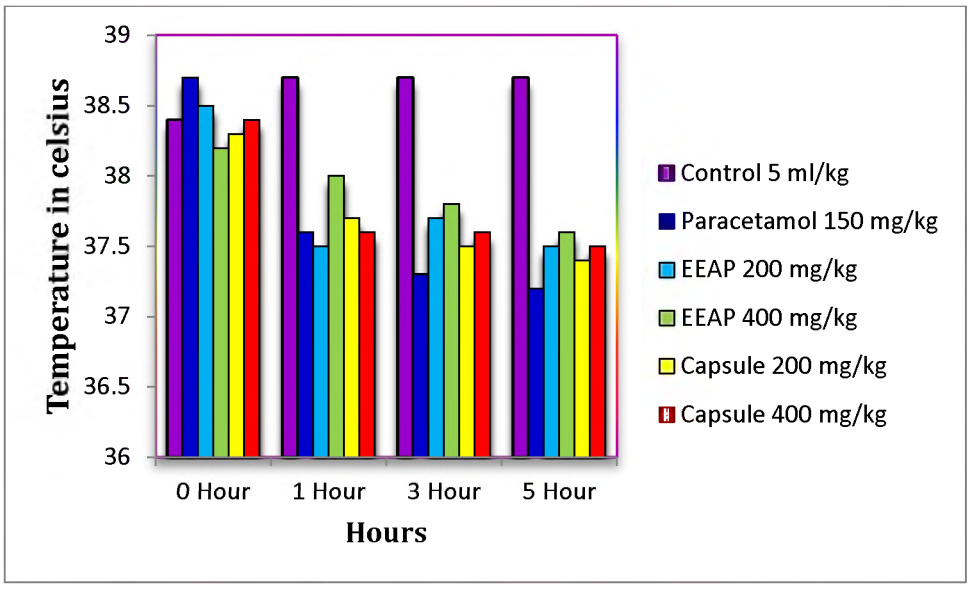


Fig.6 Comparative study of Antipyretic activity of *Andrographis paniculata*

TABLE III Antipyretic effect of ethanol and capsule form of *Andrographis paniculata*

Treatment	Rectal temperature in ^{0}C at various time intervals						
	-18h	0h	1h	2h	3h	4h	5h
Control-Saline (5 ml/kg)	37.41 ± 0.49	38.48 ± 0.87	38.76 ± 0.86	38.61 ± 1.27	38.71 ± 0.75	38.71 ± 0.79	38.71 ± 0.69
Paracetamol (150 mg/kg)	37.13 ± 0.61	38.66 ± 1.47	37.66 ± 0.36	37.46 ± 0.90	37.30 ± 0.57	37.25 ± 0.31	37.21 ± 0.16
EEAP (200 mg/kg)	37.33 ± 0.23	38.56 ± 4.23	37.68 ± 0.77	37.58 ± 0.29	37.51 ± 0.17	37.43 ± 1.01	37.29 ± 0.13
EEAP (400 mg/kg)	37.16 ± 0.19	38.21 ± 0.54	38.01 ± 0.91	37.63 ± 0.54	37.46 ± 0.54	37.37 ± 0.60	37.33 ± 0.49
Capsule (200 mg/kg)	37.22 ± 0.15	38.36 ± 0.61	37.59 ± 0.68	37.53 ± 0.33	37.43 ± 0.36	37.38 ± 0.53	37.21 ± 0.46
Capsule (400 mg/kg)	37.29 ± 0.36	38.45 ± 1.35	37.65 ± 1.40	37.53 ± 0.48	37.41 ± 0.53	37.35 ± 0.96	37. 28 ± 0.53
SE	0.047	0.058	0.182	0.178	0.227	0.227	0.132
F Test	ns	ns	*	*	*	*	*

All data were expressed in mean±SD (n=6); ns-No significance.* – Significant at P<0.05

5. DISCUSSION

Plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being. Owing to their popular use as remedies for many infectious diseases, searches for substances with antimicrobial activity in plants are frequent. Literature is flooded with compounds that have been isolated from a variety of medicinal plants. Despite this abundant literature on the antimicrobial properties of plant extracts, none of the plant derived chemicals have successfully been exploited for clinical use as antibiotics. If the isolation of antimicrobial compounds from plants is to be realistic, screening for such activities in crude extracts is the first step in identifying leads for isolation of such compounds (Sibanda and Okoh, 2007).

The Antimicrobial Susceptibility Test (AST) is used to determine the efficacy of potential antimicrobials from biological extracts against a number of diverse microbial species. Though AST methods are used to screen plant extracts for antimicrobial activity, these might not be exactly applicable to plant extracts and modifications have to be made. Agar diffusion techniques have been widely used to assay plant extracts for antimicrobial activity, though there are limitations with the technique (Ncube *et al*., 2008).

These assays are suitable for identification of leads but not effective for quantification of bioactivity (Ncube *et al*., 2008) and hence are used for preliminary or primary screening of antimicrobials.

Based on the results (i.e., the antimicrobial activity) the ethanol extract and capsule of *Andographis paniculata* exhibited varying degree of inhibitory activity against the growth both gram positive and gram negative bacteria tested. The result obtained from the study points out that the active component present in capsule form of *Andrographis paniculata* can prove to be a great remedy for

treating diseases. The mean inhibition zone for the tested bacteria ranged from (9mm-20mm) indicating a remarkable antibacterial effect when compared with Ciproflaxin the positive control, which ranged from 20mm - 25mm.

The efficacy of *Andrographis paniculata* capsule was more than its ethanolic extract. The capsule was found more active against *Klebseilla pneumonia, Shigella flexneri*, *Proteus vulgaris* and *Pseudomonas aeuriginosa* than all other species similarly the plant extract. Even though the significant antibacterial activity was observed in the other three bacteria such as *Streptococcus aureus, Staphylococcus, Escherichia coli*.

The infections caused by *Klebseilla pneumonia, E.coli, Pseudomonas aeuriginosa* and *Shigella can* be treated with ethanol extract and capsule of *Andrographis paniculata* exhibited the more inhibitory activity against the pathogen. Hence, it can be stated that *Klebseilla pneumonia, E.coli, Pseudomonas aeuriginosa* and *Shigella* were susceptible to *Andrographis*, whereas the remaining bacterial isolates were resistant and moderately susceptible. The extract of *Andrographis paniculata* have been claimed to have significant effect against the diarrhea associated with *E.coli* infections using rabbit as animal model system (Gupta *et al*., 1990). Similar results were reported by mishra *et al*., 2009 that staphylococcus is more susceptible to *Andrographis paniculata* leaf.

Generally gram positive bacteria (*Staphylococcus aureus, Streptococcus*) were more sensitive to plant extracts because of the presence of a mesh-like peptidoglycan layer which is more accessible to permeation by the extracts (Rameshkumar *et al*., 2007and Tajkarimi *et al*., 2010). The resistance of the gram negative bacteria (*Klebseilla pneumonia, Proteus vulgaris, Shigella flexneri, Escherichia coli, Pseudomonas aeruginosa*) could be attributed to its cell wall structure. Gram negative bacteria have a powerful permeability barrier,

composed of a thin lipopolysaccharide exterior membrane, which could restrict the penetration of the extruding plant extract. It has been discussed earlier that gram negative bacteria are usually more resistant to the plant originated antimicrobials and even show no effect, compared to gram positive bacteria (Tajkarimi *et al*., 2010 and Stefanello *et al*., 2008)

The compounds responsible for this antibacterial activity have not been investigated. However, preliminary phytochemical analysis of the ethanol extract revealed the presence of carbohydrates, tannins, flavonoids and saponins (Wallis 1985 and Evans 1985). The antibacterial properties of the plant may be attributed to the individual or combined effect of the above mentioned chemical groups. The findings of the present investigation offer a scientific support to the ethnomedicinal use of the plant by the traditional healers.

The Antifungal activity reported that capsule showed a great antifungal effect against *Aspergillus flavus* compared to the ethanol extract. It is all that about the compounds in the capsule which are mixed with the leaf content exhibited a zone of inhibition more than the ethanol extract

Various phytochemical compounds which are naturally present in plants as secondary metabolites have been implicated in the conferment of antifungal activities (Hostettmann and Marston, 1994; Grayer and Harborne, 1994; Osbourn, 1996; Al-Barwani and Eltayeb, 2004; Athikomkulchal *et al*., 2006; Shanker *et al*., 2007 and Fabri *et al*., 2011). The presence of some of such secondary metabolites in a significant amount in the investigated part of *A. paniculata* may have conferred the strong antifungal activity on the whole plant extracts. In this regard, higher concentration of these substances may have been responsible for a higher degree of inhibition on the tested strains.

The efficacy of ethanol extract of leaves of *Andrographis paniculata* demonstrated the presence of cell wall active antifungal agents which could lead

to the discovery and development of novel antifungal treatment therapies. Similarly such results were documented by Aplomb and Semple (2011) who reported that thick murine layer in the outer membrane prevent the entry of inhibitory substance inside the cell.

Fever is a complex physiologic response triggered by infections or aseptic stimuli. Elevation in body temperature occurs when the concentration of prostaglandin E2 (PGE2) increases within parts of the brain. Such an elevation contributes to a considerable alteration in the firing rate of neurons that control the thermoregulation process in the hypothalamus. It is now evident that most of the antipyretic drugs exert their action by inhibiting the enzymatic activity of cyclooxygenase and consequently reducing the levels of PGE2 within the hypothalamic region (Rajani *et al*., 2011).

Since antipyretic activity is commonly mentioned as a characteristic of drugs or compounds, which have an inhibitory activity on prostaglandins biosynthesis, the yeast induced hyperpyrexia in rat model was employed to investigate the antipyretic activity of *Andrographis paniculata* in the form of extract and capsules. Yeast induced pyrexia is called pathogenic fever which is due to the production of prostaglandins (PGE2) which set the thermoregulatory center at a higher temperature (Aman *et al*., 2011)

The capsule form of *Andrographis paniculata* showed more pronounced effect in lowering the hyperthermia than the ethanol extract, but found to have similar effect as the standard drug Paracetamol at 3rd hour of administration. The capsule and extract are likely to reduce pyrexia by reducing brain concentration of prostaglandin E2 especially in the hypothalamus through its action on COX-3 or by enhancement of the production of the body's own antipyretic substances like vasopressin and arginine (Jude *et al*., 2010). Many phytochemicals such as alkaloids, flavanoids, tannins and saponins in the plants materials are

responsible for their antipyretic activity. Flavonoids are known for targeting prostaglandins which are involved in the pyrexia. Hence may be the presence of large amount flavonoids in the capsule than the ethanol extract of *Andrographis paniculata* contributes to its high antipyretic effect.

The use of different plant extracts in the treatment of infections caused by various bacteria, viruses, and fungi have already been reported and recognized (Kosalec *et al.*, 2005). Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Cos *et al.*, 2006).

Hence, *Andrographis paniculata* have likely potential to be developed as safe, effective and alternative phytotherapeutic agents as antimicrobials. A further effort in regard to purification and isolation of bioactive components from their extracts is warranted for use in further research towards plant related drug discovery and development initiatives in search of newer antimicrobial therapies.

6. SUMMARY AND CONCLUSION

Since ancient time, several diseases have been treated by administration of plant extracts based on traditional medicine. It is important to note that most of the traditional medicinal plants have never been the subject of exhaustive tests as required for modern pharmaceutical compounds. Based on their traditional use for long periods of time, they are often assumed to be safe. However, research has shown that a lot of plants which are used as food ingredients or in traditional medicine have in vitro mutagenic properties. Thus, investigation of traditionally used medicinal plants is significant as a source of potential chemotherapeutic drugs and as a measure of safety for the continuous use of medicinal plants.

Andrographis paniculata is one such candidate plant, traditionally used in many Ayurvedic preparations. It is an annual and branched plant belongs to the family *Acanthaceae*. The plant is also known as the 'king of bitters'.

because it is extremely bitter in taste in every part of plant body. *Andrographis paniculata* has been treating various diseases and which are highly showing preventative effects against ailments like liver damage, infection, hyperglycemia, cancer, etc. Andrographolide is a diterpenoid lactone having a diversity of pharmacological effects specified in indigenous system of medicine.

The present study was conducted to detect the antimicrobial as well as the antipyretic efficacy of *Andrographis paniculata* leaves. It demonstrated that ethanol extract and capsule form of *Andrographis paniculata* has favourable antimicrobial and antipyretic effect. Capsule formulation showed a maximum anti -microbial and antipyretic activities that might be due to the combined effect of presence of active constituents. The antimicrobial effect of the capsule from the results highlights its future to be exploited as a potentially powerful antimicrobial agent against the both gram positive and gram negative bacteria that have made

treating nonsocial infections increasingly difficult. The antimicrobial activity results were also comparable to that of the antibiotic used as a reference. Nonetheless, further study on the phytochemistry and mechanism of action of the pure compounds are necessary to fully understand the phytochemical profile and the complex pharmacological effects of this plant

In addition to it a great number of pharmaceutical uses, *Andrographis paniculata* has some side effects like nausea, vomiting, loss of appetite which can only be seen upon overdosing. Therefore, researchers may further be undertaken to develop potent formulations consisting of *Andrographis paniculata* and its isolated molecule, andrographolide by making use of herbal drug delivery systems. All the plant part extracts and other pure phytochemicals isolated from this plant are also important to ensure its safety and eligibility as source of modern medicine.

7. REFERENCES

"Medicinal plants in Viet Nam. Manila". *World Health Organization (WHO Regional Publications* 1990). Western Pacific Series, No.3.

Agrawal, S., Tamrakar, B.P. and Paridhavi, M. 2005. "Clinically Useful Herbal Drugs" Ahuja Publishing house, Delhi, 1st Edn

Ahmad, I., Mehmood, Z., & Mohammad, F. 1998. "Screening of some Indian medicinal plants for their antimicrobial properties". *Journal of ethnopharmacology*, *62*(2), 183-193.

Akintola, A. O., Kehinde, B. D., Adeyi, R.O, Adewoyin, A. G. 2018. "Antipyretic activity of methanolic leaf extract of *Andrographis paniculata* on brewer's yeast induced pyrexia in experimental animals". *World journal of pharmacy and pharmaceutical sciences*, 7 (7):1271-1281

Al-Barwani, F. M., & Eltayeb, E. A. 2004. "Antifungal compounds from induced Conium maculatum L. plants". *Biochemical systematics and ecology*, *32*(12), 1097-1108.

Aman, A., Alzubier and Patrick Okechukwu, N. 2011. "Investigation of anti-inflammatory, antipyretic and analgesic effect of Yemeni Sid honey". *World Academy of Science*, Engineering and Technology, 80:47-52.

Arroa, R.K. 1997. "Ethnobotany and its role in the conservation and use of plant genetic resources in India". *Ethnobotany*, 9:6-15.

Athikomkulchai, S., Prawat, H., Thasana, N., Ruangrungsi, N., & Ruchirawat, S. 2006. "COX-1, COX-2 inhibitors and antifungal agents from Croton hutchinsonianus". *Chemical and pharmaceutical bulletin*, *54*(2), 262-264.

Baker, J. T., Borris, R. P., Carté, B., Cordell, G. A., Soejarto, D. D., Cragg, G. M., ... and Tyler, V. E. 1995. "Natural product drug discovery and development:

new perspectives on international collaboration". *Journal of natural products*, *58*(9), 1325-1357.

Bakht, J., Azra.G., and Shafi, M. 2012. "Antimicrobial activity of *Nicotiana tabacum* using different solvents extracts". *Pak. J.Bot.,* 44(1): 459-463.

Balmain, A., & Connolly, J. D. 1973. "Minor diterpenoid constituents of *Andrographis paniculata* Nees". *Journal of the Chemical Society, Perkin Transactions 1*, 1247-1251.

Balu, S., & Alagesaboopathi, C. 1993. "Anti-inflammatory activities of some species of Andrographis Wall. (Acanthaceae)". *Ancient science of Life*, *13*(1-2), 180.

Balunas, M.J., Kinghorn, A.D. 2005. "Drug discovery from medicinal plants". *Life Sci* 78:431-41.

Barry, A. L. 1976. "Principle and practice of microbiology". *Lea & Fabager, Philadelphia*, *3*, 21-25.

Batkhuu, J., Hattori, K., Takano, F., Fushiya, S., Oshiman, K.I. and Fujimiya, Y. 2002. "Suppression of No production in activated macrophages *in vitro* and *ex vivo* by neo andrographolide isolated from *Andrographis paniculata*", *Biological & Pharmaceutical Bulletin*, 25(9):1169–1174, 2002.

Bhavnani, S.M. and Ballow, C.H. 2000. "New agents for Gram-positive bacteria", *Curr Opin Microbil,* 3: 528-534

Borchardt, J. K. 2002. "The beginnings of drug therapy", Ancient Mesopotamian medicine. *Drug News Perspect*, 15: 187-192

Buchman, D.D., and Germerey, P.B. 1980. "Herbal Medicine, Publishing Company", New York.

Burgos, R. A., Caballero, E. E., Sanchez, N. S., Schroeder, R. A., Wikman, G.K., and Hancke, J.L. 1997. "Testicular toxicity assesment of *Andrographis*

paniculata dried extract in rats". *Journal of Ethnopharmacology*, 58(3):219–224.

Chang, R.S., Ding, L., Chen, G.Q., Pan, Q.C., Zhao, Z.L. and Smith, K. M., "Dehydro andrographolide succinic acid monoester as an inhibitor against the human immunodeficiency virus (43225)". *Proceedings of the Society for Experimental Biology and Medicine*, 197(1):59–66

Chaturvedi, G.N., Tomar, G.S., Tiwari, S.K., Singh, K.P. 1983. "Clinical studies on Kalmegh (*Andrographis paniculata* Nees) in infective hepatitis". *J Int Inst Ayurveda* 2: 208-211

Chiou, W. F., Chen, C. F. and Lin, J. J. 2000. "Mechanisms of suppression of inducible nitric oxide synthase (iNOS) expression in RAW 264.7 cells by andrographolide," *British Journal of Pharmacology*, 129(8):1553–1560.

Chiou, W.F., Lin, J.J., Chen, C.F. 1998. "Andrographolide suppresses the expression of inducible nitric oxide synthase in macrophages and restores the vasoconstriction in rat aorta treated with lipopolysaccharide". *British Journal of Pharmacology*, 125:327-334.

Chopra, R. N., Nayar, S. L., Chopra, I. C. 1980. "Glossary of Indian Medicinal Plants", New Delhi; Council for Scientific Ind.

Chopra, R.N., Nayar, S.L., and Chopra, I.C. 1956. "Glossary of Indian medicinal plants." 1st ed. Publication and Information, New Delhi, 1956.

Chopra, R.N., Nayar, S.L., Chopra, I.C. 1956. "In: Glossary of Indian medicinal plants", Council of Scientific and Industrial Research, p. 197

Cordell, C. W., Beecher, W. and Pezzuto, J. M. 1991. "Can ethno pharmacology contribute to the development of new anticancer drugs?" *Journal of Ethnopharmacology*, 32(1–3):117–133

Cordell, G.A. 2000. "Biodiversity and drug discovery a symbiotic relationship", *Phytochemistry* 55: 463-480

Corson, T. W. and Crews, C. M. 2007. "Molecular understanding and modern application of traditional medicines: triumphs and trials". *Cell*, 130: 769-774.

Cos, P., Vlietinck, A.J., Berghe, D.V., Maes, L. 2006. "Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-ofconcept'" *J Ethnopharmacol*, 106: 290–302

Cragg, G. M., & Newman, D. J. 2013. Natural products: a continuing source of novel drug leads. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1830*(6), 3670-3695.

Dastur, J.F. 1959. "Medicinal Plants of India and Pakistan." Meyer Books.

De Boer, H.J., Kool, A., Broberg, A*., et al.* 2005. " Antifungal and antibacterial activity of some herbal remedies from Tanzania", J Ethnopharmacol, 96: 461-469

de Britto Pereira, C. E., & Felcman, J. (1998). Correlation between five minerals and the healing effect of Brazilian medicinal plants. *Biological trace element research*, *65*(3), 251-259.

Deng, W. L. 1978. "Outline of current clinical and pharmacological research on *Andrographis paniculata* in China", *Newsletters Chinese Herbal Medicine*,10:27–31

Deng, W.L. 1978. " Preliminary studies on the pharmacology of the *Andrographis* product dihydro andrographolide sodium succinate", *Newsletter Chinese Herbal Medicine* 8: 26-28

Deng, W.L. 1982. "Comparison of pharmacological effect of four andrographolides". *Chinese Pharmaceutical Bulletin,* 17:195-198.

DMPRD (Divison of Medical Pants Research and Development, Department of Medical Science, ministry of public health). 1990. Handbook of Medicinal Plant for Primary Public Health. Text and journal corporation co., Ltd. Press: Bangkok; P.53

Doughari, J.H. 2006. “Antimicrobial activity of *Tamarindus indica* Linn”. *Tropical J. Pharma.* Res., 5(2): 597-603.

Duke, J.A. April 2015. “Returning to our medicinal roots (herbal medicine for alternative health care”. p. 26-33. [Online] Available from: http://www. highbeam.com/doc/1G1-73088521.html

Duraisankar, M., and Ravichandran, V. 2012. “ Antipyretic Potential of Polyherbal Ayurvedic Products”, *Asian Journal Pharmaceutical and Clinical Research*, 5 (2): 146 – 150

El Astal, Z. Y., Ashour, A. E. R. A. and Kerrit, A. A. M. 2005. “Antimicrobial activity of some medicinal plant extracts in Palestine”. *Pak. J. Med. Sci,* 21(2), 187-193.

Evans, W.C. Editor., Saunders, W.B. 1985. “Trease and evans pharmacognosy”, 12th ed. London

Fabri, R.L., Nogueira, M.S., Moreira Jdos, R., Bouzada, M.L., Scio, E. 2011. “Identification of antioxidant and antimicrobial compounds of Lippia species by bioautography”. *J Med Food*, 14, 840–846.

Fabricant, D.S., and Farnsworth, N.R., 2001. “The value of plants used in traditional medicine for drug discovery”. *Environ. Heal. Pers*. 109 (1): 69-75

Farombi, E.O. 2003. “African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents”. *African J Biotech*, 2: 662-671

Felter, H. W., & Lloyd, J. 1898. “Kings American dispensatory. Cincinnati. *OH: Eclectic Medical Publications*. (3rd rev.,2)

Grayer, R.J., Harborne, J.B.1994. “A survey of antifungal compounds from higher plants”. *Phytochemistry*, 37:19–42.

Gupta S., Yadava, J.N.S., and Tandon, J.S. 1993. "Antisecretory (antidiarrhoeal) activity of Indian medicinal plants against *E.coli* enterotoxin-induced secretion in rabbit and guinea pig teal loop models". *Pharmaceut. Biol.*, 31(3): 198-204.

Gupta, M., Shaw, B.P., and Mukerjee, A. 2008. "Studies on Antipyretic Analgesic and Ulcerogenic Activity of Polyherbal Preparation in Rats and Mice". *Intl.Journal of Pharmacology*, 4(2): 88-94.

Gupta, S. 1993. "Antisecretory (antidiarrhoeal) activity of Indian medicinal plants against *Escherichia coli* enterotoxin induced secretion in rabbit and guinea-pig ileal loop models". *International Journal of Pharmacognosy*, 31:198-204.

Gupta, S., Choudhry, M.A., and Yadava, J.N.S. *et al.*, 1990. "Antidiarroeal activity of diterpenes of *Andrographis paniculata* (Kal-Megh) agent *Escherichia coli* enterotoxin *in vivo* models. Intl. J. Crude Drug Res., 284: 273-283

Gupta, S.1990. "Antidiarrhoeal activity of diterpenes of *Andrographis paniculata* (kalmegh) against *Escherichia coli* enterotoxin in *in vivo* models". International Journal of Crude Drug Research, 28:273-283.

Gurib-Fakim, A., 2006. "Review Medicinal plants: Traditions of Yesterday and drugs of tomorrow". Mol. Aspect. Med., 27: 1- 93s.

Hajra, P.K., Mudgal, V. 1997 "Plant Diversity Hot Spots in India: An Overview. Calcutta".

Hammer, K.A., Carson, C.F. and Riley, T.V. 1999. "Antimicrobial activity of essential oils and other plant extract". *J. app. Microbiol.*, 86: 985-990

Handa, S.S. and Sharma, A. 1990. "Hepatoprotective activity of andrographolide from *Andrographis paniculata* against carbon tetrachloride". *Indian J. of Medicinal Research*, 92(b):276-283

Hassanali. 2003. "An investigation of Antimicrobial Compounds for Immunomodulating and Antiadhesion properties". *Immunology and Infectious Disease and research Laboratory*, PhD Thesis, 01-186.

Hazeena Begum, V., and Velavan, S. 2011. "Lipids regulating activity of *Asparagus racemosus* root in young and aged rats". *Indian Journal of Gerontology*, 25(3): 273-285.

Hooker, J.D. 1885. "Flora of British India", L. Reeve & Co. LTD. Ashford, Kent, 4

Hostettmann, K., Marston, A.1994. "Search for new antifungal compounds from higher plants". *Pure & Appl Chem*, 66, 2231–2234

Hosztafi, S. 1997. "The discovery of alkaloids". Pharmazie., 52: 546-550.

Hung, S.K., Hung, L.C., Kuoetal, C.D. 2010. "Andrographolide sensitizes Ras-transformed cells to radiation *in vitro* and *in vivo*". *International Journal of Radiation Oncology Biology Physics*, 77(4):1232–1239

Ighodaro Igbe, Ozolua, R.I., Okpo S.O. and Osahon Obasuyi. 2009. "Antipyretic and analgesic effects of the aqueous extract of the Fruit pulp of *Hunteria umbellata K Schum* (*Apocynaceae*). *Tropical Journal of Pharmaceutical Research,* 8(4): 331-336

Ignacimuthu, S., and Ayyanar, M. 2009. "Herbal medicines for wound healing among tribal people in Southern India". Ethno botanical and Scientific evidences. *International Journal of Applied Research in Natural Products,* 2 (3): 29-42.

Iruretagoyena, M. I., Sep´ulveda, S. E., Lezana, J. P., *et al*., 2006. "Inhibition of nuclear factor-κB enhances the capacity of immature dendritic cells to induce antigen-specific tolerance in experimental auto immune encephalo myelitis". *The Journal of Pharmacology and Experimental Therapeutics*, 318(1):59–67.

Iruretagoyena, M. I., Tobar, J. A., Gonz´alez, P. A. *et al.*, 2005 "Andrographolide interferes with Tcell activation and reduces experimental autoimmune encephalomyelitis in the mouse". *The Journal of Pharmacology and Experimental Therapeutics*, 312(1):366–372

Jain, M.P., Koul, S.K., Dhar, K.L., Atal, C.K., 1980. "Novelnor-harmal alkaloid from *Adhatoda vasica*. *Phytochemistry* 19:1880–1882.

Jarukamjorn, K., Kondo, S., Chatuphonprasert, W., Sakuma, T., Kawasaki, Y., Emito, N. 2010. "Gender-associated modulation of inducible CYP1A1 expression by andrographolide in mouse liver". *Eur J Pharm Sci* 39: 394-401

Jiang, C.G., Li, J.B., Liu, F.B., Wu, T., Yu, M. and Xu, H.M. 2007. "Andrographolide inhibits the adhesion of gastric cancer cells to endothelial cells by blocking E-selectin expression".*Anticancer Research*, 27(4B):2439–2447

Joseph, A., Di Masi. 2003. "The price of innovation: new estimates of drug development costs". *J. of Health Econ*, 22: 151-185.

Joshi, B., Sunil, B. and Anuja, S. 2009. "Antibacterial property of different medicinal plants: *Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum* and *Origanum majorana*". *Kathmandu University Journal of Science*, Engineering and Technology, 5(1): 143-150.

Kaiser, H. 2008. Von der Pflanze zur Chemie–die Frühgeschichte der „Rheumamittel ". *Zeitschrift für Rheumatologie*, *67*(3), 252-262..

Kandya, A. K. 2005. "Cultivation of Some Medicinal Plant Species and Requirement of Seeds", Pharmacognosy Magazine, 1 (2): 38-44.

Kanjilal, P. B., Bordoloi, S., Kalita, R., Burman, P. and Singh, R. 2002. "Cultivation practices for Kalmegh (*Andrographis paniculata*) and Spiderling

(Boerhaavia diffusa) in Assam India". *Recent progress in medicinal plants*, 5, 175-180..

Kanokwan, J. and Nobuo, N. 2008. "Pharmacological aspects of *Andrographis paniculata* on health and its major diterpenoid constitute andrographolide". *J of Health Sci*., 54 (4): 370-381.

Kataky, A., and Handique, P.J. 2010. "Micropropagation and screening of antioxidant potential of *Andrographis paniculata* (Burm. f) Nees. *Journal of Hill Agriculture*., 1(1): 15-20

King Spalding, L.L.P. 2006. "Andrographolide derivatives to treat viral infections", *US 20060333785.*

Kinghorn, A. D., Pan, L., Fletcher, J. N. and Chai, H. 2011. "The relevance of higher plants in lead compound discovery programs". *J. Nat. Prod.,* 74: 1539- 1555.

Kosalec, I., Pepeljnjak, S., Kustrak, D. 2005. "Antifungal activity of fluid extract and essential oil from anise fruits (*Pimpinella anisum L., Apiaceae*)". *Acta Pharm* 55: 377–385

Kruse, P. R. 2007. Geschichte der Pharmazie.: Von der Fruhen Neuzeitbiszur Gegenwart- by Rudolf Schmitz. *Centaurus*, 2(49): 182-183

Kunwar, R. M., Shrestha, K. P., and Bussmann, R. W. 2010. "Traditional herbal medicine in far-west Nepal: a pharmacological appraisal". *Journal of Ethnobiology and Ethnomedicine,* 6(35):1–18.

Lavanya, G., and Brahmaprakash. G.P. 2011. " Phytochemical screening and antimicrobial activity of compounds from selected medicinal and aromatic plants". *Int. J. Sci & Nature*, 2(2): 287-291.

Li, J., Cheung, H.Y., Zhang, Z., Chan, G.K.L., and Fong, W.F. 2007. "Andrographolide induces cell cycle arrest at G2/M phase and cell death in

HepG2 cells via alteration of reactive oxygen species". *European Journal of Pharmacology*, 568 (1–3):31–44.

Li, L. 2000. "Opportunity and challenge of traditional Chinese medicine in face of the entrance to WTO (World Trade Organization)". *Chin. Inform. trad. Chin. Med.*, 7: 7–8

Lin, F. L. Wu, S. J. Lee, S. C., and Ng, L. T. 2009. "AntioxSidant, anti oedema and analgesic activities of *Andrographis paniculata* extracts and their active constituent androsgrapholide". *Phytotherapy Research*, (23)7, 958–964.

Lin, T.P., Chen, S.Y., Duh, P.D., Chang, L.K., and Liu, Y.N. 2008. "Inhibition of the Epstein-Barr virus lytic cycle by andrographolide". *Biological & Pharmaceutical Bulletin*, 31(11):2018– 2023

Liu, Z. T. Wang, L. L. Ji, and Ge, B. X. 2007. "Inhibitory effects of neo andrographolide on nitric oxide and prostaglandin E2 production in LPS-stimulated murine macrophage". *Molecular and Cellular Biochemistry*, 298(1-2):49–57.

Madav, S., Tripathi, H. C., Tandan, and Mishra, S. K. 1995. "Analgesic, antipyretic and antiulcerogenic effects of andrographolide". *Indian Journal of Pharmaceutical Sciences*, 57(3):121– 125

Mahesh, B., and Satish, S. 2008. "Antimicrobial activity of some important medicinal plant against plant and human pathogens". *World J. Agri. Sci.,* 4(S): 839-843.

Maiti, K., Gantait, A., Mukherjee, K., Saha, B.P., and Mukherjee, B.K. 2006. "Therapeutic potentials of andrographolide from *Andrographis paniculata* :are view". *Journal of Natural Remedies*, 6(1):1–13.

Manikam, S. D. and Stanslas, J. 2009. "Andrographolide inhibits growth of acute pro myelocytic leukaemia cells by inducing retinoic acid receptor-independent cell differentiation and apoptosis". *The Journal of Pharmacy and Pharmacology*, 61(1):69–78.

Matsuda, T., Kuroyanagi, M., Sugiyama, S., Umehara, K., Ueno, A. and Nishi, K. 1994. "Cell differentiation-inducing diterpenes from *Andrographis paniculata* nees". *Chemical & Pharmaceutical Bulletin*, 42(6):1216–1225

Matsuda, T., Kuroyanagi, M., Sugiyama, S., Umehara, K., Ueno, A., Nishi, K. 1994. "Cell- differentiation– inducing diterpenes from *Andrographis paniculata* Nees". *Chem. Pharm. Bull*. 42(6): 1216 - 1225.

Mishra, K., Dash, A.P., Swain, B.K. and Dey, N. 2009. "Antimalarial activities of *Andrographis paniculata* and *Hedyotis corymbosa* extracts and their combination with curcumin". *Malaria Journal*, 8(1), article26

Mishra, S.K., Sangwan, N.S., and Sangwan, R.S. 2007. "*Andrographis paniculata* (Kalmegh): A review". *Pharmacog. Rev*., 1: 283-289

Monroe, S., and Polk, R. 2000. " Antimicrobial use and bacterial resistance". *Curr Opin Microbiol*, 3: 496-501

Morgan, K. 2002. "Medicine of the Gods: Basic Principles of Ayurvedic Medicine [http://www. compulink.co.uk/mandrake/ayurveda.html]

Mukherjee, P. K. 2002. "Quality Control on Herbal Drugs", *Business Horizons*, NewDelhi, India, 1st edition

Nadkarni, A.K. 1954. Nadkarni's "Indian Materia Medica" 1, 1st ed. *Popular Book Depot.* Bombay.

Nair, R., Kalariya, T. and Sumitra, C. 2005. "Antibacterial activity of some selected Indian medicinal flora". *Turk. J. Biol*. 29:41-47.

Nanduri, S., Nyavanandi, V. K., Thunuguntla, S. S. R. *et al*., 2004. "Synthesis and structure-activity relationships of andrographolide analogues as novel cytotoxic agents". *Bioorganic & Medicinal Chemistry Letters*, 14(18):4711–4717

Ncube, N.S., Afolayan, A.J. and Okoh, A.I. 2008. "Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends". *Afr. J. Biotechnol.* 7 (12):1797-1806.

Nostro, A., Germano, M.P, Angelo,V.D., Marino,A. and Cannatelli,M.A. 2000. "Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity". *Letters in Applied Microbiology*, 30: 379-384.

Okokon, J.E. and Paul Nwafor. 2010. "Antiinflammatory, analgesic and antipyretic activities of ethanol root extract of *Croton zambesicus*", *Pak. J. Pharm. Sci.*, 23(4): 385-392.

Olabanji, S. O., Makanju, O. V., Ceccato, D., Buoso, M. C., Haque, A. M. I., Cherubini, R., & Moschini, G. (1997). PIGE-PIXE analysis of medicinal plants and vegetables of pharmacological importance. *Biological trace element research*, *58*(3), 223-236

Osbourn, A.E. 1996. "Preformed antimicrobial compounds and plant defense against fungal attack". *Plant Cell*, 8, 1821–1831

Oudhia. 2009. *P.Andrographis paniculata* Née. Purdue Horticulture Aromatic and Medicinal Plant Database. Available from http:// www. hort. purdue. edu/newcrop/Crop Fact Sheets/andrographis.html.

Patwardhan, B. 2005. "Ethnopharmacology and drug discovery". *J. Ethnopsharmacol.*, 100:50 52.

Periyasamy Gomathi, Upal Kanti Mazumder and Malaya Gupta. 2011. "Antipyretic potential of *Galega pupurea* root". *Intl. Research Journal of Pharmacy*, 2(11): 151-152.

Perry, L.M.,1980 "Medicinal plants of East and Southeast Asia: attributed properties and uses", Cambridge: MIT Press

Pharmacopoeia of the People's Republic of China. 1997. Vol.1 (ed). Beijing, Chemical Industry Press.

Pinner, R., Teutsch, S., Simonsen, L., Klug, L., Grabers, J., Clarke, M. and Berkelman, R. 1996. "Trends in infectious diseases mortality in the United States". *J.Am. Med. Assoc*, 275: 189-193, 1996

Poolsup, N., Suthisisang, C., Prathanturarug, S., Asawamekin, A. and Chanchareon, U. 2004. "*Andrographis paniculata* in the symptomatic treatment of uncomplicated upper respiratory tract infection: systematic review of randomized controlled trials". *Journal of Clinical Pharmacy and Therapeutics*, 29(1):37–45.

Principe, P., 2005. "Monetising the pharmacological benefits of plants", US Environmental protection Agency, Washington, D.C.

Puri, A., Saxena, R., Srivastava, V. and Tandon, J.S. "Immuno stimulant agents from *Andrographis paniculata*," *Journal of Natural Products*, 56(7):995– 999.

Qin, L. H., Kong, L., Shi, G. J., Wang, Z. T. and Ge, B. X. 2006. "Andrographolide inhibits the production of TNF-α and interleukin-12 in lipopolysaccharide-stimulated macrophages: role of mitogen-activated protein kinases", *Biological & Pharmaceutical Bulletin*, 29(2):220–224

Rahman, N.A., Furuta, T., Kojima, S.K., Tabane, K. and Ali-Mohd, M. (1999). "*In vitro* and *in vivo* study revealed that malarias medicinal plants *Piper sarmentosum*, A. paniculata and *Tinospora crispa* produce considerable anti malarial effect". *J.Ethnopharmacol.*, 64: 249-254.

Rahman, N.N.N.A., Furuta, T., Kojima, S., Takane, K., and AliMohd, M. 1999. "Anti malarial activity of extracts of Malaysian medicinal plants". *Journal of Ethnopharmacology*, 64, 3: 249– 254.

Rajagopal, S., Kumar, R.A., Deevi, D.S., Satyanarayana, C. and Rajagopalan, R. 2003. "Andrographolide, apotential cancer therapeutic agent isolated from

Andrographis paniculata". *Journal of Experimental Therapeutics and Oncology*, 3(3):147–158

Rajani, G. P., Deepak Gupta, Sowjanya, K. and Sahithi, B. 2011. "Screening of antipyretic activity of aerial parts of *Nelumbo nucifera gaertn* in yeast induced pyrexia", *Pharmacologyonline*, 1: 1120-1124.

Rajkumar, J. S., Sekar, M. G. and Mitra, S. K. 2007. "Safety and efficacy of oral HD-03/ES given for six months in patients with chronic hepatitis B virus infection". *World Journal of Gastroenterology: WJG*, *13*(30), 4103.

Ram, V.J. 2001. "Herbal preparations as a source of hepatoprotective agents",*Drug News and Perspectives*, 14(6):353–363

Rameshkumar, K.B., George, V., Shiburaj. 2007. "Chemical constituents and antibacterial activity of the leaf oil of *Cinnamomum chemungianum*", Mohan et Henry. *Journal of Essential Oil Research*, I19 (1):98-100.

Rana, A.C. and Avadhoot, Y. 1991. "Hepatoprotective effects of *Andrograhphis paniculata* against carbon tetrachloride induced liver damage,"*Archives of Pharmacal Research*, 14(1):93–95.

Rastogi, R.P. and Mehrotra, B.N. 1993. "Compendium of Indian medicinal plants", New Delhi: CDRI and Publication and Information Directorate. 3: 1980– 1984.

Rocha, A.B.Da., Lopes, R.M., and Schwartzman, G. 2001. "Natural products in anticancer therapy". *Current Opinion in Pharmacology*, 1(4):364–369.

Sah, N., Khan, M., & Vohora, S. B. 1991. "Antimicrobial activity of some medicinal plants extracts on Escherichia coli, Salmonella paratyphi and Shigella dysenteriae". *Fitoterapia*, *62*(3), 221-228.

Samantaray, S., Rout, G.R., Das, P. 2001. "Heavy metal and nutrient concentration in soil and plants growing on a metalliferous chromite minespoil", *Enviro rech*, 22:1147-54. http://dx.doi.org/

Sankar Anand, Subhadra Devi, Arunprasath, B., Subageetha, A. and Anusha, C. H. 2010. "Boiled milk induced pyrexia in rabbits- antipyretic activity Vernonia cinerea roots". *IJPSR*, 2(1): 127-131.

Saxena, S., Jain, D.C., Bhakuni, R.S. and Sharma, R.P. 1998. "Chemistry and pharmacology of *Andrographis* species". *Indian Drugs*, 35(4): 58-467.

Saxena, S., Jain, D.C., Bhakuni, R.S., Sharma, R.P. 1998. "Chemistry and pharmacology of *Andrographis* species", Indian Drugs, 35: 458-467.

Service, R.F. 1995. "Antibiotics that resist resistance". *Science*, 270: 724-727

Seubsasana, S., Pientong, C., Ekalaksananan, T., Thongchai, S. and Aromdee, C. 2011. "A potential andrographolide analogue against the replication of herpes simplex virus type 1 in vivo cells". *Medicinal Chemistry*, 7(3):237–244.

Shanker, K.S., Kanjilal, S., Rao, B.V., Kishore, K.H., Misra, S., Prasad, R.B. 2007. "Isolation and antimicrobial evaluation of isomeric hydroxy ketones in leaf cuticular waxes of *Annona squamosa*". *Phytochem Anal*, 18, 7–12.

Sharma, A., Shanker, C., Tyagi, L. K., Singh, M. and Rao, Ch. V. 2008. "Herbal Medicine for Market Potential in India: An Overview". *Academic Journal of Plant Sciences*, 1 (2): 26-36.

Sharma, P.V. 1983. Charka Samhita Ed. "Chankhambhia Sorientalia", 2, Varanasi,

Sheeja, K. and Kuttan, G. 2007. "Activation of cytotoxic Tlymphocyte responses and attenuation of tumor growth *in vivo* by *Andrographis paniculata* extract and andrographolide". *Immunopharmacology and Immunotoxicology*, 29(1):81–93.

Shen, Y. C., Chen, C. F. and Chiou, W. F. 2000. "Suppression of rat neutrophil reactive oxygen species production and adhesion by the diterpenoid lactone andrographolide". *Planta Medica* 66(4):314–317

Sherwin, E.R., Branen, A.L., Davidson, P.M. and Salminen, S. 1990. "FoodAdditives", Marcel Dekker, NewYork, USA.

Shi, G., Zhang, Z., Zhang, R. *et al*., 2012. "Protective effect of andrographolide against concanavalin A-induced liver injury". *Naunyn's Schmie debergs Archives of Pharmacology*, 385, (1):69–79

Shi, M.D., Lin, H.H., Lee, Y.C., Chao, J.K., Lin, R.A., and Chen, J.H. 2008. "Inhibition of cell-cycle progression in human colorectal carcinoma Lovo cells by andrographolide". *Chemico-Biological Interactions*, 174(3):201–210.

Shukla, B., Visen, P.K.S., Patnaik, G.K. and Dhawan. 1992. "Choleretic effect of andrographolide in rats and guinea pigs," *Planta Medica*,58(2):146–149

Sibanda, T. and Okoh, A.I. 2007. "The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents", *Afr.J. Biotechnol.* 6 (25):2886-2896

Simic, M. G. 1988. "Mechanisms of inhibition of free-radical processes in mutagenesis and carcinogenesis*". Mutation Research*, 202(2):377–386.

Singha P.K., Roy S. and Dey S. 2003. "Antimicrobial activity of *Andrographis paniculata*". *Fitoterapia*, 74: 692-694.

Singha, P. K., Roy, S. and Dey, S. 2007. "Protective activity of andrographolide and arabinogalactan proteins from *Andrographis paniculata* Nees. Against ethanol-induced toxicity in mice". *Journal of Ethnopharmacology*, 111(1):13–21.

Siripong, P., Kongkathip, B., Preechanukool, K., Picha, P., Tunsuwan, K. and Taylor, W. C. 1992. "Cytotoxic diterpenoid constituents from *A. paniculata* Nees leaves". *Journal of Scientific Soceity of Thailand*,18:187–194

Sneader, W. 2005. "Drug Discovery: A History", (Eds., Soejarto, D.D., Gyllenhaal, C., Fong, H., Xuan, L.T., and Hiep), Wiley.

Standard of ASEAN herbal medicine 1993. 1. Jakarta, ASEAN Countries.

Stefanello, M.É.A., Cervi, A.C., Ito, I.Y, Salvador, M.J., Wisniewski, A. and Simionatto, E.L. 2008. "Chemical composition and antimicrobial activity of essential oils of *Eugenia chlorophylla* (*Myrtaceae*). *Journal of Essential Oil Research*, 20(1):75-78.

Stuffness, M. and Douros , J. 1982. "Current status of the NCI plant and animal product program". *J Nat Prod*, 45: 1-14

Suebsasana, S., Pongnaratorn, P., Sattayasai, J., Arkaravichien, T., Tiamkao, S. and Aromdee, C. 2009. "Analgesic, Antipyretic, Anti-Inflammatory and Toxic Effects of Andrographolide Derivatives in Experimental Animals". *Archives of pharma research*, 32(9):1191-1200

Tajkarimi, M.M., Ibrahim, S.A. and Cliver, D.O. 2010 "Antimicrobial herb and spice compounds in food". *Food Control*. 21(9):1199-1218.

Tang, L.I.C., Ling, A.P.K., Koh, R.Y., Chye, S.M. and Voon, K.G.L. 2012. "Screening of anti-dengue activity in methanolic extracts of medicinal plants". *BMC Complementary and Alternative Medicine*, 12(3):1–10

Thai herbal pharmacopoeia 1995. Bangkok, Prachachon Co. Vol. 1.

Thamlikitkul, V., Theerapong, S. and Boonroj, P. *et al*., 1991. "Efficacy of *Andrographis paniculata*, nees for pharyngotonsillitis in adults". *Journal of the Medical Association of Thailand*, 74(10): 437–442

Tiwari, D.N. 2000. "Report of the Task Force on Conservation and Sustainable Uses of Medicinal Plants", New Delhi: Bull Planning Commission, Govt. of India: 23.

Trivadi, N.P. and Rawal, U.M. 2001. "Hepatoprotective and antioxidant property of *Andrographis paniculata* (Nees) in BHC induced liver damage in mice. *Indian J.Exp. Biol.*, 39(1): 41-46.

Trivedi, N. P. and Rawal, U. M. 2001. "Hepatoprotective and antioxidant property of *Andrographis paniculata* (Nees) in BHC induced liver damage in mice". *Indian Journal of Experimental Biology*, 39(1):41–46,2001

Tumwine, W. 2011. "Implementation of the framework convention on tobacco control in Africa: Current Status of Legislation". *Int. J. Environ.,Res. and Public Health*, 8: 4312-4331.

Unschuld, P. U. 1986. "Medicine in China: A History of Pharmaceutics", University of California Press, Berkeley, and London: 181.

Verma, N. and Vinayak, M. 2008 "Antioxidant action of *Andrographis paniculata* on lymphoma". *Molecular Biology Reports*, 35(4):535–540.

Verpoorte, R., Contin, A., and Memelink, J. 2002 *Phytochemistry Reviews*, 1 (1): 13–25.

Visen, P. K. S., Shukia, B., Patnaik, G. K. and Dhawan, B. N. 1993. "Andrographolide protects rat hepatocytes against paracetamol-induced damage". *Journal of Ethno pharmacology*, 40(2):131–136

Vlietinck, A.J., Van Hoof, L., Tott, J., *et al.,* 1995. "Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties".*J. Ethnopaharmacol*, 46:31-47

Voravuthikunchai, S. P. and Limsuwan, S.2006. "Medicinal plant extracts as anti-*Escherichia coli*O157:H7agents and their effects on bacterial cell aggregation". *Journal of Food Protection*,69(10):2336–2341

Wallis, T.E., 1985. Editor. Text book of pharmacognosy. New Delhi: CBS Publishers and Distributor: 252.

WHO. 2003. "Fact Sheet No. 134: Traditional Medicine" available at http://www.who.int/mediacentre/factsheets/2003/fs134/en/

Wiart, C., Kumar, K., Yusof, M.Y., Hamimah, H., Fauzi, Z.M. and Sulaiman, M.2005. "Antiviral properties of ent-labdene diterpenes of *Andrographis paniculata* Nees inhibitors of herpes simplex virus type 1". *Phytotherapy Research,* 19(12) 1069– 1070

Xu, Y., Marshall, R.L. and Mukkur, T.K.S. 2006. "An investigation on the antimicrobial activity of *Andrographis paniculata* extracts and andrographolide *in vitro*". *Asian Journal of Plant Sciences*, 5(3):527–530.

Zaidan, M.R., NoorRain, A., Badrul, A.R., Adlin, A., Norazah, A. and Zakiah, I. 2005. "*In vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method". *Tropical Biomedicine*, 22(2):165–170.

Zhangand, X.F. and Tan, B. K. H. 2000. "Anti-diabetic property of ethanol extract of *Andrographis paniculata* in streptozotoc in diabetic rats". sActa *Pharmacological Sinica*, 21(12):1157–1164.

APPENDIX

IAEC CERTIFICATE

Avinashilingam Institute for Home Science and Higher Education for Women
(Deemed to be University under Category 'A' by MHRD, Estd. u/s 3 of UGC Act, 1956)
Re-accredited with 'A' grade by NAAC, Recognised by UGC under Section 12 B
Coimbatore – 641 043, Tamil Nadu, India

Dr.P.R.Padma
Dean, School of Biosciences
Professor and Head
Department of Biochemistry, Biotechnology and Bioinformatics

CERTIFICATE

This is to certify that the project title "Evaluation of Antipyretic activity of NK (decoction) and comparison with NK (capsule) using animal model" has been approved by the IAEC.

Name of the chairman, IAEC
Dr.P.R Padma

Name of the CPCSEA Nominee
Dr. C. Gunasekaran

Signature with date

Chairman, IAEC: 30/1/19

CPCSEA Nominee: 30/1/19