

Analysis of Biochemical and Phytochemical Parameters of
***Chromolaena odorata* (L.) R.M. King & H. Rob. and**
***Adhatoda vasica* Nees**

Bindhu, V.
(Reg. No. 11PBO02)

A Thesis Submitted to
Avinashilingam Institute for Home Science and Higher Education for
Women
Coimbatore – 641 043

In Partial Fulfillment of the Requirements for the
Degree of Master of Science in Botany

April 2016

**Analysis of Biochemical and Phytochemical Parameters of
Chromolaena odorata (L.) R.M. King & H. Rob. and
Adhatoda vasica Nees**

**Bindhu, V.
(Reg. No. 11PBO02)**

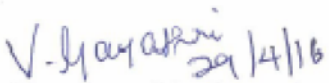
**A Thesis Submitted to
Avinashilingam Institute for Home Science and Higher Education for
Women
Coimbatore – 641 043**

**In Partial Fulfillment of the Requirements for the
Degree of Master of Science in Botany**

April 2016


Signature of the

Head of the Department


Signature of the Supervisor

Acknowledgement

I wish to record my profound sense of gratitude to **Padma Sri Thiru. P.R.Krishnakumar**, Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for giving me an opportunity to study in this esteemed university.

I gratefully record my sincere thanks to **Dr. (Tmt.) Premavathy Vijayan**, M.Sc., M.Ed., Dip. Spl.Edn., M.Phil., Ph.D., Vice Chancellor (i/c), Avinashilingam Institute for Home science and Higher Education for Women, Coimbatore for facilities provided and constant encouragement to complete the research work.

I am much obliged to express my sincere thanks to **Dr. (Tmt.) A.Venmathi**, M.Sc., Dip.Ed., M.Phil., Ph.D., Registrar (i/c), Avinashilingam Institute for Home science and Higher Education for Women, Coimbatore for giving this golden opportunity to undertake this course in this university.

I extend my thanks to **Dr. (Tmt.) A. Parvathi**, M.Sc., Dip. Ed., M.Phil., Ph.D., Dean, Faculty of Science, Avinashilingam Institute for Home science and Higher Education for Women, Coimbatore for her encouragement throughout the course of study.

I record my sincere thanks to **Dr. (Tmt.) A.Vijayalakshmi**, M.Sc., M.Phil., Ph.D., Professor and Head, Department of Botany, Avinashilingam Institute for Home science and Higher Education for Women, Coimbatore for the help and guidance given by her.

I pay my obeisance to god for having bestowed with an opportunity and privilege of being guided by **Dr. (Tmt.) V. Gayathri**, M.Sc., Ph.D., Assistant Professor, Department of Botany, and Avinashilingam Institute for Home science and Higher Education for Women, Coimbatore during the course of this

study. I cannot express in words my heartfelt thanks and noble indebtedness for valuable guidance, noble ideas, immense patience, keen interest, cordial treatment and constant encouragement with kind advice throughout the period of investigation.

I record my gratitude and sincere thanks to all the **staff members of Department of Botany** for their inspiration and constant encouragement evinced throughout the course of this study.

Last but not least, I whole heartedly bestow my gratitude and gratefulness to my **affectionate parents V. Balakrishnan and V. Bhargavi, my lovable husband N. Sunil Kumar, my lovable son N.S. Abhinav, my lovable sister V. Babitha, my lovable brother V. Binukrishnan, my family members, my dear most friends and my well-wishers** for their constant encouragement, good support, prayer, loving care and kind help rendered in various ways throughout the period of this investigation.

Above all I thank God Almighty for showering his blessings for the successful completion of my studies.

CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
	LIST OF PLATES	
	LIST OF TABLES	
	LIST OF FIGURES	
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	9
3	MATERIALS AND METHODS	17
4	RESULTS AND DISCUSSION	35
5	SUMMARY AND CONCLUSION	48
	REFERENCES	49

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
1	Chlorophyll a, Chlorophyll b and Total Chlorophyll content of the medicinal plants	37
2	Protein content of the medicinal plants	39
3	Carbohydrate content of the medicinal plants	41
4	Preliminary phytochemical analysis of <i>Chromolaena odorata</i>	44
5	Preliminary phytochemical analysis of <i>Adhatoda vasica</i>	46

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO.
1	Chlorophyll a, Chlorophyll b and Total Chlorophyll content of the two medicinal plants	37
2	Protein content of the two medicinal plants	39
3	Carbohydrate content of the two medicinal plants	41

LIST OF PLATES

PLATE NO.	TITLE	PAGE NO.
1	Morphology of the plant - <i>Chromolaena odorata</i>	21
2	Morphology of the plant - <i>Adhatoda vasica</i>	25
3	Leaf powder of <i>Chromolaena odorata</i>	31
4	Leaf powder of <i>Adhatoda vasica</i>	31

CHAPTER I

INTRODUCTION

Plants that are used for therapeutic purpose are termed as medicinal plants or herbs. Plants which have one or more of its organic substance that are used for the therapeutic purpose are called medicinal plants. Those plants which have healing properties are termed as medicinal plants or herbs. Medicinal plants can be simply classified as trees, shrubs, woody perennials, annuals, biennials and climbers (Anubha Aroara, 2013). Plants which have one or more of its organ containing substances that can be used for the curative purpose are called medicinal plants (Sofowara, 1993).

India has a rich source of medicinal plants. India recognizes more than 3000 plant species which have medicinal values. Herbal medicines have become more popular in the treatment of many diseases due to popular confidence that green medicine is safe, easily available and less side effect. Use of traditional medicine cures a wide range of diseases in developing countries (Bhumi and Savithramma, 2014). Medical herbalism is the practice of healing with medicinal plants. Modern western treatment is different from medical herbalism.

Plant extracts have great possible as antimicrobial compound against microorganisms. The medicinal value of plants lies in the bioactive compounds such as alkaloids, flavonoids, tannin and phenolic compounds that produce a specific physiological action on the human body (Hill, 1952).

The increasing use of plant extracts in food, cosmetic and pharmacological industries suggests that in order to extract active compounds, a methodical study of medicinal plants is important (Arunkumar *et al.*, 2010).

The use of conventional medicines holds a great secure as an easily available source as efficient medicinal agents to heal a wide range of ailments among the people predominantly in humid developing countries like India. People consume several plants or plant derived formulations to cure helminthic infections and healing of wounds (Debashisha Panda *et al.*, 2010).

Plants are major source of herbal medicines and the presence of secondary metabolites in plants concerned them for many therapeutic activities (Sachin Kumar *et al.*, 2011). Also, the plants have provided a source of stimulation for new drug compounds, as plant derived

medicines have made large assistance to human health and well being. A major part of the total inhabitants in developing countries still use traditional folk medicine obtained from plant resources. The people of Indian villages use crude plants as medicine since Vedic period and still continue.

Plants have played an important role as various medicinal agents since ages. The knowledge of Indian medicinal plants and their use in Ayurvedic and Unani system of medicine have led to many Scientific Investigations and Researches throughout the world. Researches on the Indian medicinal plants have been going on far more than half a century. Various active principles have been isolated from the plants and many of them play a dominating role in the modern therapy. Medicinal herbs have been in use in one form or another, under indigenous systems of medicine like Ayurveda, Siddha and Unani. India, with its traditional background, needs to increase its share in the world market. But unlike China, India has not been able to capitalize on this herbal wealth by promoting its use in the developed world, despite their renewed interest in herbal medicines. Such herbal medicines will find speedy access into those countries (Arvind Dangi *et al.*, 2015).

It is well known that traditional herbal medicines existed before the application of the modern scientific methods to health care; and even today, majority of the world population depends on herbal health care practices. Exploring traditional herbal medicines in the context of modern science is the need for most favourable and proper consumption of traditional plant drugs (Asvin Godghate and Rajaram Sawant, 2013).

Plants have played a significant role in maintaining human health and refining the quality of human life for thousands of years. The use of plants as medicines is as old as human civilization itself and out of about 2, 58,650 species of higher plants reported from the world; more than 10% are used to cure ailing communities (Shinwari, 2010). Many of the existing medicinal system such as Ayurveda, Unani, Homeopathy, Naturopathy, Sidha and other alternative medicinal system have been utilizing plants as effective medicines to cure many harmful diseases (Prasad *et al.*, 2011). The world health organization (WHO) has predicted that 80% of the earth's inhabitant relied on traditional medicine for their primary health care needs and most of these therapies involved the use of plant extract or their active compounds (Bruneton, 1995).

Many earlier workers have done considerable exploration on the medicinal vegetation of India, which eventually contributed a wide variety of active constituents and plant drug to modern therapy. Natural product drug materials are a diverse group of product ranging from parts of plants, through simple extract, to isolated active constituents. The definition encompasses a wide range of natural materials, which are important for their therapeutic activity or as pharmaceutical adjuvant. India is sitting on a gold mine of well-recorded and traditionally well-practiced knowledge of herbal medicine. This country is perhaps the largest producer of medicinal herbs and is rightly called the botanical garden of the world. There are very few medicinal herbs of commercial importance, which are not found in this country. India formally recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the Third World countries (Arvind Dangi *et al.*, 2015)

Plant based antimicrobials represent a vast available source. The use of plant extract for medicinal treatment has become popular when people realized that the effective life span of antibiotic is limited and over prescription and misuse of traditional antibiotics are causing microbial resistance (Alam *et al.*, 2009). At present, nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and their extracts govern in homeopathic or ayurvedic medicines (Murugesan *et al.*, 2011).

Medicinal plants are finding their way into pharmaceuticals, cosmetics and nutraceuticals. Plants have been given Western Pharmacopoeia about 7000 different pharmacologically important compounds and a number of top advertising drugs of modern times eg. Quinine, taxol, camptothecine etc (Tshibangu *et al.*, 2002).

People have always been interested in plants for their medicinal properties. Since ancient times, people practiced herbal medicine as a magical or religious healing art. Throughout the ages, people have turned for healing to herbal medicine. All cultures have folk medicinal traditions that include the use of plants and plant products. Tribes are using their traditional knowledge system to cure different diseases. They use plants as a source of drug. Herbal medicines have good values in treating many diseases including infectious diseases, hypertension, etc (Lady Jane G. Morilla *et al.*, 2014).

Medicinal plants are now attractive, more widely used by people all over the world. People understand the gentle strength of these natural remedies. Doctors and scientist are

validating the wisdom of traditional use. Several natural remedies can be used to cure infirmity rather than just mask the symptoms (Riaz Ullah *et al.*, 2013) .

Chromolaena odorata

Chromolaena odorata is a hastily growing perennial herb. It belongs to the family Asteraceae. It is a multi-stemmed shrub, grows upto 2.5m (100 inches) tall in open areas. It is an unknown, obnoxious and destructive weed. The plant has the ability to rejuvenate from the roots (Shetonde Mihigo *et al.*, 2015). It is native to Central and South America, but it has become established pantropically (Zachariades *et al.*, 2009).

This weed was probably introduced into Nigeria about 50 years ago and found along road-sides, waste and fallow lands. *Chromolaena odorata* was first identified in Central America and Vietnam. It is a diffused scrambling herb that is mainly a weed of plantation crops and field of Southern Asia and West Africa (Nyananyo, 2006).

The aqueous extract and the decoction from leaves of the plant have been used throughout Vietnam for the treatment of soft tissue wounds and burns. Various parts of *C.odorata*, i.e. leaf, stem and root, were extracted with water, ethanol, methanol and hexane and used for treatment for skin infections (Omoregie *et al.*, 2014).

The plant possess greater therapeutic value in wound healing process, which can be reasonable by the presence of various phytochemicals on different solvent extracts of the leaves of *Chromolaena odorata*. *Chromolaena odorata* leaves possess strong haemostatic properties and may be useful in wound healing and management of bleeding problems. It is a persistent plant and nuisance in nature (Akomas and Ijioma, 2014).

In Malaysia, *C. odorata* is known as, “pokok kapal terbang”, “pokok Jerman”, “rumput Jepun” or “rumput Siam”. It thrives in most soils and is a creative weed found in plenty on open wasteland and along roadsides. It is used as an antibacterial, antiplasmodic, antiprotozoal, antitrypanosomal, antifungal, antihypertensive, antiinflammatory, astringent, diuretic and hepatotropic agent (Iwu *et al.*, 1999).

It is also applied topically as an antidote against the sting from the spine of the common sea catfish. An aqueous decoction of the roots is used as an antipyretic and analgesic remedy, and a leaf extract with salt is used as a gargle for relief of sore throat and cold. In Vietnam and other tropical countries, fresh leaves or decoction of the leaves are used for

treatment of leech bite, soft tissue wounds, burn wounds, skin infection and dento-alveolitis. (Nuha Binthi Cheiman, 2010).

Chromolaena odorata is inhabitant to North America from Florida and Texas to Caribbean, and has been introduced to South America, tropical Asia, West Africa and parts of Australia. A decoction of the leaf is used as a cough medicine and as an ingredient with lemon grass. And also with guava leaves, are used for the treatment of malaria. Other traditional medicinal uses include anti-diarrheal, astringent, antispasmodic, anti hypertensive, anti-inflammatory, diuretic tonic, antipyretic and heart tonic (Kigiga *et al.*, 2013).

In the southern part of Nigeria, the leaves of *Chromolena odorata* are used for wound healing, skin infection and also to stop bleeding. Some specific phenolic compounds have also been isolated from the plant. The medicinal values of plants lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body. The nutritive value of *Chromolaena odorata* includes high crude protein (CP), low fibre and low extractable phenolic contents that has good potential for feeding livestock. In India, various studies have been carried out to extract various plant materials for screening antimicrobial compounds but much attention has not been focused on *Chromolaena odorata* (Sukanya *et al.*, 2011).

ADHATODA VASICA

Adhatoda vasica is a small evergreen plant belonging to the family Acanthaceae. *Adhatoda vasica* grows in plains of India and in the lower Himalayas, up to a range of 1000 metre above sea level. This plant is also cultivated in other tropical areas. It grows well in low moisture areas and dry soils. The Vasaka plant is perennial, evergreen and highly branched with unpleasant smell and bitter taste. The plant lives in multiple season and retains its leaves throughout the year. It is a shrub that grows upto a height of 2.5m (Jayapriya and Gricilda, 2015).

Adhatoda vasica is commonly known as Malabar nut, and locally known as Adulsa, is found in many regions of India and throughout the world. This plant has several uses in traditional Ayurveda. The leaves, flowers, fruits and roots are widely used for treating cold, cough, whooping cough, chronic bronchitis and asthma. It is used as sedative and expectorant. It is an antispasmodic herb. Boiled with sesame oil, it can be used to heal ear infections and arrest bleeding. Boiled leaves are used to treat rheumatic pain and to relieve the pain of

urinary tract infections. It also possess abortifacient properties. It is used in some parts of India to stimulate uterine contractions, thus speeding childbirth (Agwane *et al.*, 2015)

The leaves, root, flowers and stem bark of the plant are used for medicinal purposes. Extracts of the leaves of *Adhatoda vasica* are extensively used in cough, asthma, bronchitis, tuberculosis, inflammation and allergic reaction (Santosh Kumar Singh *et al.*, 2014).

Several active constituents have also been isolated from different parts of the plant. Its leaves are used for the treatment of jaundice in Bengal. Leaves contain several alkaloids like vasicine and vasicinone responsible for medicinal activity (Ajay Kumar Gautam and Shubhi Avasthi, 2013).

All the plant parts normally possess expectorants, antiseptic and antispasmodic properties and also used to treat cough and cold, asthma, dysentery and rheumatic pain. The leaves of *Adhatoda vasica* contain potent phytochemicals responsible for their antimicrobial effect. The essential and the fragrant volatile oils extracted from plant leaves are rich in borneol which is a tremendous antimicrobial compound (Santoyo *et al.*, 2005).

The plant is medicinally important and used in many ayurvedic formulations. It is a highly accepted plant used in Ayurvedic system of medicine for the treatment of various ailments of respiratory systems like bronchitis, asthma and it is also used in the treatment of malaria and dysentery. It shows powerful anti-inflammatory activity and *Adhatoda vasica* was usually used by midwives at the time of delivery, because of its uterotonic activity. *Adhatoda vasica* posses anti-implantation activity, so *Adhatoda* should not be used during pregnancy. It has anti-inflammatory and analgesic properties also. *Adhatoda vasica* also possess antioxidant, hepatoprotective, sedative, antispasmodic and anthelmintic properties. This plant also possesses antimicrobial activity, antidiabetic activity, wound healing effect, infertility and antiulcer activity (Jayram patel *et al.*, 2014)

In Ayurvedic medicine, *Adhatoda vasica* is used for a large number of disorders including, leprosy, blood disorders, heart troubles, fever, vomiting, loss of memory, leucoderma, jaundice, tumors, mouth troubles, sore-eye and gonorrhoea. This herb is known for its antispasmodic, expectorant and blood-purifying qualities (Yarapa *et al.*, 2013).

The drug is employed in different forms such as fresh juice, decoction, infusion and powder. It is also given as alcoholic extract and liquid extract or syrup. The leaf juice is stated to cure diarrhoea, dysentery and glandular tumor and the plant is an emmenagogue. The

powder is reported to be used as poultice on rheumatic joints as counter-irritant on inflammatory swelling, on fresh wounds, urticaria and in neuralgia. This plant contains alkaloids, tannins, flavonoids, terpenes, sugars and glycosides. Chemical constituents of *Adhatoda vasica* leaves are vasicine, vasicinol, vasicol, vasicinone, adhatonine and pyrroloquinazoline alkaloids etc. Young plant contain the quinazoline alkaloids (vasicine, 7-hydroxyvasicine, vasicinolone, 3-deoxyvasicine, vasicol, vasicoline, vasicolinone, adhatodine, anisotine), betaine, steroids, carbohydrate and alkanes. In the flowers, triterpenes (a-amirine), flavonoids (Apigenin, astragaline, kaempferol, quercetin, vitexin) have been found (Sunita Maurya and Dhananjay Singh 2010)

Adhatoda vasica is a well-known plant drug in Ayurveda and Unani medicine (Manjunath, 1948). The plant has been used in the indigenous system of medicine in India for over 2000 years (Atal, 1980). Infectious diseases remain the important cause of death worldwide and infections due to antibiotic resistant microorganisms have become more widespread in recent years (Inderjit Kaur *et al.*, 2012).

It is well known that herbal medicines are in great demand as primary healthcare remedy because of great effectiveness and no side effects (Narula *et al.*, 2000). *Adhatoda vasica* is an important medicinal herb used by Naga tribes in India for curing parasitic intestinal worm. Quinazoline alkaloids present in the leaves are well-known as active principles (Jeya and Veerakumari, 2013). In modern medicine, the active ingredients were found to be vasicine, oxyvasicine and vasicinone. The alkaloid present in vasaka is the active ingredient for expelling sputum from the body (Nandre *et al.*, 2012).

Adhatoda vasica Nees. Leaf (vasaka), known as *Vasa* in Ayurveda, is an important drug prescribed for malarial fever, fever caused by pitta and kapha, chronic fever, intrinsic hemorrhage, cough and asthma, leprosy, skin diseases and piles. It is used as an expectorant and abortifacient, antimicrobial, antitussive and anticancer. Important chemical constituents of leaf include pyrroloquinazoline alkaloids, vasicine, vasicol, adhatonine, vasicinone, vasicinol, vasicinolone, vasicine was reported to have bronchodilatory, respiratory stimulant and uterine stimulant effect. Vasicinone was shown to have bronchodilatory, weak cardiac stimulant and antianaphylactic action (Savithamma *et al.*, 2014).

In Ayurvedic preparations, *Vasaka* leaf juice (*Vasa swarasa*) is incorporated in more than 20 formulations including *Vasarishta*, *Mahatiktaka ghrita*, *Triphalaghrita*, *Vasavaleha*,

Vasakasava, Mahatriphalaghrita, Panchatiktaghritaguggulu and Panchatikta ghrita. Classical methods for extracting *Vasaka* juice is an elaborate process which involves subjecting a bolus of crushed fresh leaf to heat. This method is not applicable in large scale extraction of juice for commercial purpose. Hence, in the commercial manufacture of the formulations containing *Vasaka* juice (*Swarasa*), to prepare *swarasa*, modified methods are being adopted. The leaves of *Adhatoda vasica* contain powerful phytochemicals that are responsible for their antimicrobial effect (Chanu *et al.*, 2014).

The main objectives of the present study are :

- 1) To analyse the biochemical parameters in the freshly collected leaf sample of *Chromolaena odorata* and *Adhatoda vasica*.
- 2) To screen for the phytochemical parameters in the two test plants.

CHAPTER II

REVIEW OF LITERATURE

The available literature pertaining to the biochemical parameters and phytochemical aspects relevant to the present investigation has been reviewed and presented in this chapter. Manjunath (1948) suggested that *Adhatoda vasica* is a well known plant drug in Ayurvedic and Unani medicine.

Hill (1952) explained that the medicinal values of plants lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body. Egunjobi (1969) reported that the plant *Chromolaena odorata* is locally called “*bienqua*” among the Ijaws in the Niger Delta region of Nigeria where it is believed to possess healing potentials for wounds and treatment of pile ailment.

Sajise *et al.* (1974) reported that the economic value of *Chromolaena odorata* is low. In recent decades, it has become a serious pest in the humid tropics of South East Asia, Africa and Pacific Islands. Zaika (1975) reported that the natural antimicrobials can be derived from plants. Nadkarni *et al.* (1976) suggested that the leaves, roots and flowers of the plant contain the alkaloid vasicine, which is responsible for the persistent bronchodilatation and an essential oil which is the main ingredient responsible for the expectorant action.

In a field study, Holm *et al.* (1977) have suggested that the plant is one of the worst alien invasive plant species in the humid tropics and sub-tropics of the old world, where it is a menace to agriculture, human health and biodiversity. Its prolific regeneration from seeds frustrates attempts to eliminate the plant manually. *Chromolaena odorata* is one of the most troublesome invasive species in Nigeria and in many tropical countries.

Bhat *et al.* (1978) reported that the chemical compounds found in *Justicia adhatoda* plant includes essential oils, fats, resins, sugar, gum, aminoacids, proteins, vitamin ‘C’ etc.

Atal (1980) have found that the plant has been used in the indigenous system of medicine in India. For several years, *Adhatoda* leaves have been used extensively in Ayurvedic medicine primarily for respiratory disorders including cough, cold, asthma, bronchitis etc. Infectious diseases remain the leading cause of death worldwide and infections due to antibiotic resistant microorganisms have become more widespread in recent years.

Metwally and Ekejuba (1981) suggested that in the southern part of Nigeria, the leaves are used for wound dressing, skin infection and to stop bleeding. Pandita (1983) reported that the *Adhatoda vasica* plant is recommended for a variety of ailments such as bronchitis, asthma, fever and jaundice. Fukamiya & Lee, 1986; Hui *et al.* (1986) have performed pharmacological tests using the ethanol extract from *Justicia reptans*.

Hui *et al.* (1986) have suggested that lignans obtained from *Justicia pectoralis* are cytotoxic to leukemia and solid tumour cell lines. A field study was conducted by Jain and Defilipps (1991) to investigate the use of plant extensively in the treatment of asthma, cough, bronchitis, tuberculosis, joint pain, lumbar pain, sprains, eczema, malaria, rheumatism, swellings, venereal diseases, etc.

Gill (1992) suggested that the leaves of *Chromolaena odorata*, when chewed have also been claimed to alleviate headache and toothache. The plant *Adhatoda vasica* has been used in the treatment of cold, cough, pneumonia, fever, jaundice, catarrh, whooping cough and asthma in homeopathy (Asolkar *et al.*, 1992). The side effects of drugs available today compel the discovery of new pharmaco-therapeutic agents from medicinal plants (Cordell, 1993).

Siddiqui and Hussain (1993) suggested that the paste of roots of *Adhatoda vasica* is mixed with sugar and used for treatment of acute nightfall in Sitapur District, Uttar Pradesh, India. Biller *et al.* (1994) reported that *Chromolaena odorata* contain the N-oxides of 5-pyrrolizidine alkaloids: 7- and 9-angeloylretronecine, intermedine, rinderine etc.

Vlietinck *et al.* (1995) have explained that various plant parts such as leaves, bark, fruits, roots and seeds are used in treatment of various diseases. It had been reported that aqueous and methanolic extracts from plants used in allopathic medicines were potential sources of antiviral, anti tumour and antimicrobial agents.

Kasamota *et al.* (1995) have studied the selection of crude plant extracts for screening programs is potentially more successful in initial steps than the pure compounds. Essential oils and plant extracts have been screened for their potential use as alternative remedies for the treatment of many infectious diseases.

Pushpangadan *et al.* (1995) reported that the plant leaves are used for checking postpartum haemorrhage and urinary trouble. Salalamp *et al.* (1996) suggested that the *Adhatoda vasica* possess antiperiodic, astringent, diuretic, purgative properties and is also used as an expectorant in addition to liquifying sputum.

Chromolaena odorata has been reported to possess anti-inflammatory, astringent, diuretic and hepatoprotective activities (Weniger and Robinean, 1988). Baquar (1997) has reported that the leaves are used for treating respiratory disorders and the juice from its leaves is used as remedy in treating diarrhoea and dysentery. The alkaloids- vasicine and vasicinone present in the leaves possess respiratory stimulant activity.

Nath *et al.* (1997) have found that 70% of the pregnant women in the Gora village of Lucknow (Uttar Pradesh, India) use the leaves of *Justicia adhatoda* to induce abortion. In a field experiment, Joshi & Joshi (2000) have shown that, flower, fruits and roots of *Justicia adhatoda* are extensively used for treating cold, cough, chronic bronchitis and asthma.

Claeson *et al.* (2000) suggested that *Justicia adhatoda* is a well-known plant drug in Ayurvedic and Unani medicines. It has been used for the treatment of various diseases and disorders, particularly for the respiratory tract ailments like bronchitis, asthma, tuberculosis, cold and cough (Sharma *et al.*, 1992).

In a field study, Phan *et al.* (2001) reported that *Chromolaena odorata* is also applied topically as an antidote against the sting from the spine of the common sea catfish. An aqueous decoction of the roots is used as an antipyretic and analgesic remedy, and a leaf extract with salt is used as a gargle for sore throats and colds. In Vietnam and other tropical countries, fresh leaves or decoction of the leaves are used for treatment of leech bite, soft tissue wounds, burn wounds, skin infection and dento-alveolitis.

Mathew Masoli *et al.* (2002) suggested that to increase the economic wealth and to improve the distribution of resources between and within countries represent important priorities to enable better health care that has to be provided.

Sheetz (2002) have explained that, hyperglycemia and hyperlipidemia are the two important characters of *Diabetes mellitus* in which, diabetic patients experience various vascular complications such as, coronary heart disease, diabetic nephropathy and diabetic neuropathy.

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action (Jayakar and Suresh, 2003).

Afolayan (2003) have revealed that the economically important medicinal plants, not only provide raw materials for pharmaceuticals, perfumery, flavor and cosmetic industries, but, also protect and cure human against certain diseases. Anderson Badami *et al.* (2003) studied the effect of phytochemical and biological potential of other species of *Justicia*. The number of pregnancies among treated women were significantly less than that of the control group. These results indicate the abortifacient nature of the roots of these plants.

In a field study, Wild *et al.* (2004) have shown that the occurrence of diabetes is higher in men than in women and a notable increase in the proportion of people suffering from diabetes with more than 65 years of age is also reported. Wild *et al.* (2004) has conducted a study and evaluated that the number of people suffering from diabetes worldwide is increasing at an alarming rate. It is predicted that about 366 million people are likely to be diabetic by the year 2030.

Bedi *et al.* (2004) suggested that the aqueous extract of the leaves could be used for the treatment of abdominal and cervical pain and also for the treatment of wounds as a local antiseptic agent (Bamba *et al.*, 1993).

Suksamrarn *et al.* (2004) reported that the root extract of *Chromolaena odorata* contains triterpenes, poriferasterol, octadecane, butyrospermolacetate, bis (2ethylhexyl)

phthalate, chrysophanol and physcion. Gupta *et al.* (2004) and Watcho *et al.* (2004) have revealed that the crude alkaloids from *Chromolaena odorata* leaves act as the most implicated reproductive toxicant.

An experiment was carried out to determine the virucidal effect of leaf extracts of *Justicia reptans* on HIV, which was attributed to two glycosylated flavonoids that have not yet been identified. Compounds of this chemical class have been previously reported to display anti-HIV properties including reverse transcriptase or integrase inhibition, but this is the first time that they are described as virucides (Kumar *et al.*, 2005)

Adhatoda vasica is used as an expectorant and as a bronchodilator (Taydae and Patil, 2005; Mishra and Broker, 2009; Singh *et al.*, 2010; Mahajan, 2007; Dey *et al.*, 2009; Venkataswamy *et al.*, 2010 and Muhbubur Rahman *et al.*, 2013). Kpoviessi *et al.* (2006) have reported that the alcoholic extracts from the aerial parts of *Justicia anselliana* showed allelopathic effects on cowpea plants .

A study was carried out in *Adhatoda zeylanica* to determine the biochemical components such as alkaloids, flavonoids, saponins, vasicine, vasicinone, vasicinolone, proteins, carbohydrates etc. that are found mostly in leaves (Muhammad *et al.*, 2006).

Adriana *et al.* (2006) reported that the medicinal plant is infected by various fungal pathogens that are responsible for causing reduction in biochemical components of plant parts. Hence, there is a need to study disease severity of these plants and to know the reasons responsible for decreasing medicinal properties of these plants.

Mohanta *et al.* (2007) has conducted a study on herbal medicinal drugs that have become increasingly popular and their use is widespread. There is a need to develop alternative drugs for the treatment of infectious diseases from medicinal plants.

Dogra *et al.* (2008) suggested that the invasion of plant species also alters the physicochemical properties of soils in the invaded areas that become nutrient-rich, which generally helps in the growth of invasive species.

Cui *et al.* (2009) suggested that the essential oils obtained from *Chromolaena odorata* have been scientifically validated for antibacterial, insecticidal and insect repellent activities.

Igboh *et al.* (2009) revealed that *Chromolaena odorata* leaves contain high amount of total carbohydrate, crude fiber and protein that is rich in essential amino acids such as histidine and phenylalanine. Furthermore, the leaves have been reported to contain alkaloids, cyanogenic glycosides, flavonoids (aurone, chalcone, flavone and flavonol), saponins and tannins.

Kaushik (2009) has reported that medicinal plants constitute important components of flora and are widely distributed in different regions of India. Leaves are used to treat cough, asthma, fever, tuberculosis, piles, jaundice, bleeding gum, etc.

Karthikeyan *et al.* (2009) have reported that *Adathoda vasica* can be used as an expectorant and antispasmodic and also act as a bronchodilator. Ramaya and Jayakumara (2009) suggested that the decoction of plant is given in cold and in rheumatism.

Panda *et al.* (2010) reported that the methanolic extract prepared from the plant was found to possess antihelmintic and wound-healing properties. Sampath Kumar (2010) carried out experiment with *Adathoda vasica* and found the use of plant in speed delivery during childbirth and the use of root, bark, leaves and flower extract in treatment of bronchial, asthmatic and pulmonary infections.

Adnan *et al.* (2010) have explained that the various preparation of leaves are used for curing bleeding, haemorrhage, skin diseases, wounds, headache and leprosy in Southeast Asia.

Sampath *et al.* (2010) have studied Indian traditional herbs *Adhatoda vasica* and its medicinal application. Vasaka is a well-known herb in indigenous systems of medicine for its beneficial effects, particularly, in bronchitis. Vasaka leaves, bark, fruit and flowers are useful in the removal of intestinal parasites. Vasaka herb is used for treating cold, cough, chronic bronchitis and asthma. The decoction of its root and bark in doses of 30 grams twice or thrice a day for 3 days can be given for this purpose. The juice of its fresh leaves can also be used thrice a day.

Ignacimuthu *et al.* (2010) have evaluated anti myco- bacterial activity of two natural alkaloids, vasicine acetate and 2-acetyl benzylamine, isolated from Indian shrub *Adhatoda vasica* leaves. In folk medicine, *Adhatoda vasica* leaves is used to treat asthma and cough. The leaves of *Adhatoda vasica* were powdered and extracted with hexane, ethyl acetate and methanol.

Seema *et al.* (2010) suggested that the chemical metabolites obtained from leaves are used as medicine against bronchitis, leprosy, blood disorders, ear diseases, thirst, asthma, fever, vomiting, loss of memory, leucoderma, jaundice, tumors, etc.

Kedare and Singh (2011), Das *et al.* (2014) have studied the effect of plant on the treatment of various degenerative disorders like mutagenesis, carcinogenesis, cardiovascular disturbances and ageing. Ibegbulam *et al.* (2011) revealed that *Eupatorium odoratum* may be used as an inhibitor of corrosion in aluminium alloy products .

Anyasor *et al.* (2011) investigated that the chemical compounds produced by this plant play a role in inhibiting the growth of pathogenic microorganisms and also the leaf extract contains coumarins, tannins, steroids, saponins, terpenoids, terpenes, flavonoids and cardiac glycosides. Flower extract contains flavonoids (isosakuranetin, persicogenin, 5,6,7,4'-tetramethoxy flavanone and 4'-hydroxy-5,6,7-trimethoxy flavanone) and flavones (acacetin and luteolin). Alisi *et al.* (2011) have explained the importance of *Chromolaena odorata* in livestock nutrition, improvement of soil fertility and its potential use as a pesticide.

Hung *et al.* (2011) have explained that *Chromolaena* is an important weed in tropical and subtropical areas extending from West, Central and Southern Africa to India, Sri Lanka, Bangladesh, Laos, Cambodia, Thailand, Southern China, Taiwan, Indonesia, etc. *Chromolaena odorata* has been used as an effective therapy against diarrhoea, malarial fever, tooth ache, diabetes, skin diseases, dysentery and has been demonstrated to have anti-inflammatory activity (Vaisakh and Pandey, 2012)

Rak *et al.* (2012) demonstrated that *Eupatorium odoratum* has the ability to absorb oil from oily waste water and also in connection with selected bacterial strains can effectively degrade the absorbed oil. *Eupatorium odoratum* may thus find use in remediation of contaminated soils.

CHAPTER III

MATERIALS AND METHODS

In the present study, fresh and dry leaf sample of two different medicinal plants were used. The medicinal plants were *Chromolaena odorata* and *Adhatoda vasica*. A study was carried out on various biochemical parameters and phytochemical aspects using different solvent extracts.

COLLECTION OF PLANT SAMPLES

The fresh leaves of *Chromolaena odorata* and *Adhatoda vasica* were obtained from Elembulassery Village in Palakkad district of Kerala .

MORPHOLOGY OF THE PLANTS

Chromolaena odorata

Systematic position

Kingdom	-	Plantae
Class	-	Eudicots
Order	-	Asterales
Family	-	Asteraceae
Genus	-	<i>Chromolaena</i>
Species	-	<i>C. odorata</i>

Description

- It is a perennial, diffuse and scrambling shrub which grows to 3-7m in height, it forms dense tangled bushes when growing in the open land. It occasionally reaches its maximum height of 6m (as a climber on other plants). Its stem branches freely with lateral branches developing in pairs from axillary buds. The older stems are brown and woody near the base, tip and young shoots are green and succulent in nature (Plate 1).
- The plant can regenerate from the roots. In favorable conditions, the plant can grow more than 3 cm per day. The root system is fibrous and does not penetrate beyond 20-30 cm in moist soils.
- The leaves alternate, opposite or whorled around the stem. Sometimes they are situated at the base of the stem (radical or rosulate) or in groups. The leaves are petiolated. The leaves can be simple with smooth margins or the margins can be toothed, lobed or variously dissected to such an extent that the leaves are actually compound with numerous leaf segments. The leaves are flaccid, membranaceous, velvety, pubescent, deltoid, ovate, acute and 3-nerved. The leaves are entire when young, base obtuse or subtruncate, but, shortly decurrent. Petiole of the leaves is slender and 1-1.5 cm long, leaf blade mostly 5-12cm long. The leaves are bracteate and the bracts are slender in nature. Involucre of about 4-5 series of bracts pale with green nerves. Leaves are 4–10 cm long by 1–5 cm wide (up to 4 x 2 inches).
- The flower heads are borne in terminal corymbs of 20-60 heads on all stem branches. The flowers are white or pale bluish and form masses covering the whole surface of the bush. Florets are alike (disc florets) and pale purple to dull white in colour. The style of the flower extending about 4mm beyond the apex of the involucre. It spread radiately. Receptacle of the flower is very narrow. Florets about 20-30 or a few more, 10-12mm long, ovarian portion 4mm long. Corolla of the flower appears in a slender trumpet form, pappus of dull white hairs are present. The white

to pale pink tubular flowers are in panicles of 10 to 35 flowers that form at the ends of branches of plant.

- Seeds are achenes and are somewhat hairy, seeds is glabrous or nearly so. Seeds of *chromolaena odorata* are small and 3-5 mm long, 1mm wide and weight of seed about 2.5 mg in nature. They are mostly spread by the wind, but can also cling to fur, clothes and machinery, enabling long distance dispersal. Seed production is about 80,000 to 90,000 per plant. Seeds need light to germinate. The plant can regenerate from the roots. In favorable conditions, the plant can grow more than 3 cm per day.

Distribution

This plant species is native to Central America, was probably introduced into Africa as an ornamental. It is reported to be present in different countries of Sub-Saharan Africa, in Kenya, Malawi, Nigeria, Rwanda, Uganda, Cameroon, Zimbabwe and Zambia. It is also reported to be widely dispersed throughout the humid and sub-humid tropics in Central and South America, Asia and Africa .

Chromolaena odorata is a species of flowering shrub in the sunflower family, Asteraceae. It is native to North America, from Florida and Texas to Mexico and the Caribbean, and has been introduced to tropical Asia, West Africa, and parts of Australia.

It has become highly hostile persistent throughout many tropical countries. It was introduced to Botanical Gardens of Dacca (India), Java and Peradeniya (Sri Lanka) in the 19th century and for ornamental reasons in Southern Africa in the early 20th century. In West Africa, the plant was accidentally introduced with forestry seeds in Nigeria in 1947 .

Medicinal uses

- *Chromolaena odorata* is used as a traditional medicine in various parts of the world such as, Indonesia, Thailand, Malaysia and parts of Africa including Nigeria.
- The young leaves are crushed and the resulting liquid can be used to treat skin wounds. The boiled roots of *Chromolaena odorata* are used for urinary retention and leaves are used to cure malaria, gonorrhoea, ulcers and blood in urine. The kani tribal

of Kouthalai in Tirunelveli hills used the leaf extract to cure skin diseases, poison bites, wounds and rheumatism.

- This plant possess anthelmintic, antimalarial, analgesic, anti-inflammatory, antipyretic, antispasmodic, antioxidant, antigonorrhoeal, antimycobacterial, insecticidal, fungicidal, wound healing, diuretic, blood coagulation and antibacterial properties.
- In traditional medicine of Thailand, the plant is used for the treatment of wounds, rashes, diabetes and as insect repellent.
- The phytoprostane compound chromomoric acid C-I has been identified from *Chromolaena odorata* as a strong inducer of the activity of the transcription factor NFE2L2 (Nrf2), a master regulator of a range of genes with defensive, anti-inflammatory and detoxifying functions.
- The plant possess ethno-pharmacological, fungicidal, nematicidal importance and its use as a fallow species and as a soil fertility improvement plant in the slash and burn alteration system of agriculture, has contributed to its continued use and spread in Nigeria.



PLATE -1

MORPHOLOGY OF THE PLANT - *CHROMOLAENA ODORATA*

Adhatoda vasica

Systematic position

Kingdom	-	Plantae
Class	-	Eudicot
Order	-	Lamiales
Family	-	Acanthaceae
Genus	-	<i>Adhatoda</i>
Species	-	<i>A. vasica</i>

Description

- *Adhatoda vasica* is an evergreen shrub of 1-3 feet in height with many long opposite climbing branches. The older stem is greyish green, warty and woody. The stem is herbaceous above and woody below and the wood is soft (Plate 2).
- The leaves of the plant are simple, large, opposite, petiolate and exstipulate. The leaves measure 7-19 cm long and 4-7 cm wide and lance shaped. The margin is crenate with acuminate apex and there are 8-10 pair of lateral veins. The leaves become greenish brown when dried and have a bitter taste. The leaves have smell similar to strong tea.

- Flowers of *Adhatoda vasica* are white, pink or purple in colour. The flowers are 2-lipped, creamy-white with purple colour streaks on the lower lip of the flowers. They are arranged on a dense leafy spike or panicles.
- The flowers are small, irregular, zygomorphic, bisexual and hypogynous. *Adhatoda vasica* produces its flowers and then prune-shaped fruits through the month of August to November. It has capsular four seeded fruits.
- The fruits of *Adhatoda vasica* are compressed capsule, the capsule is small, clavate, and longitudinally channelled containing four globular seeds. The seeds of the plant are orbicular compressed.

Distribution

- *Adhatoda vasica* is a perennial shrub growing on the plains of India and in the lower Himalayas upto a range of 1000 meters above sea level. The plant is cultivated in other tropical areas. It grows well in low moisture area and dry soils.
- *Adhatoda vasica* commonly grown in waste places and distributed throughout India and also in Maharashtra especially in Konkan region. Besides India, it is found in Myanmar, Srilanka and Malaya.

Medicinal uses

- The leaves, roots and flowers of *Adhatoda vasica* also called vasa or vasaka, were used extensively in Traditional Indian Medicine for thousands of years to treat respiratory disorders such as asthma.
- *Adhatoda vasica* is considered useful in treating bronchitis, tuberculosis and other lung and bronchiole disorders.
- A decoction of the leaves may be used as an herbal treatment for cough and other symptoms of cold.

- The soothing action helps to treat irritation in throat and the expectorant will help loosen phlegm deposits, in the airway which makes *Adhatoda vasica*, a good remedy for sore throat.
- A poultice of the leaves may be applied to wounds for their antibacterial and anti-inflammatory properties and also to joints to relieve pain.
- It has been used to control both internal and external bleeding such as peptic ulcers, haemorrhoids and bleeding gums.
- In Ayurvedic medicine, it is used for a large number of disorders including leprosy, blood disorders, heart troubles, fever, vomiting and loss of memory, leucoderma, jaundice, tumours, mouth troubles, sore eye and gonorrhoea.
- *Adhatoda vasica* is known for its antispasmodic, expectorant and blood purifying qualities.



PLATE - 2

MORPHOLOGY OF THE PLANT - *ADHATODA VASICA*

Biochemical parameters

The following biochemical parameters were observed in the leaves of both the medicinal plants used for the present study.

- Chlorophyll
- Protein
- Carbohydrate

Estimation of Chlorophyll content

Chlorophyll 'a', 'b' and total chlorophyll were analyzed following the method of Arnon (1949).

Materials required

Analytical grade acetone was diluted to 80% acetone.

Procedure

- One gram of freshly cut leaf sample was taken in a clean mortar.
- The leaf bits were ground to a fine pulp with the addition of 20ml of 80 % (w/v) acetone.
- The mixture thus obtained was centrifuged at 5000rpm for 5 minutes.
- The supernatant was transferred to 100ml volumetric flask. This procedure was repeated until the residue became colourless.
- The washing was collected and the volume was made upto 100ml in the flask with acetone.
- The absorbance of the solution was read in a spectrophotometer at 645 and 663nm against the solvent blank (80% acetone).

Calculation

The amount of chlorophyll present in the extract was calculated (mg chlorophyll/gm tissue) using the formula,

$$\begin{aligned} \text{❖ mg chlorophyll 'a' / gm tissue} &= 12.7A_{663} - 2.69A_{645} \times \frac{v}{1000 \times w} \\ \text{❖ mg chlorophyll 'b' / gm tissue} &= 22.9A_{645} - 4.68A_{663} \times \frac{v}{1000 \times w} \\ \text{❖ mg total chlorophyll / gm tissue} &= 20.2A_{645} + 8.02A_{663} \times \frac{v}{1000 \times w} \end{aligned}$$

where,

A= Absorbance at specific wave length

V= Final volume of chlorophyll extract in 80% acetone

W=Fresh weight of the tissue

1. Estimation of protein (Lowry *et al.*, 1951)

Principle

The blue colour developed by phosphomolybdic phosphotungstic components in the folin –ciocalteau reagent by the aminoacids, tyrosine and tryptophan present in the protein and the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured by Lowry's method.

Materials required

- Two percent sodium carbonate in 0.1N sodium hydroxide (Reagent A)
- 0.5 percent copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in 1% potassium sodium tartarate (Reagent B)
- Alkaline copper solution : Mix 50 ml of reagent A and 1ml of reagent B prior to use (Reagent C)
- Folin –Ciocalteau Reagent (Reagent D)

- Protein solution (stock standard) : Accurately 50mg of bovine serum albumin was weighed and dissolved in distilled water and made upto 50 ml in a standard flask.
- Working standard: Ten ml of stock solution was diluted to 50ml with distilled water in a standard flask. One ml of this solution contains 200mg protein.

Procedure

Extraction of protein samples

Extraction was carried out with buffers. About 500mg of the sample was weighed and ground well with a pestle and mortar in 5-10ml of phosphate buffer, centrifuged and supernatant was used for protein estimation.

Estimation of protein

- About 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard were pipetted out into a series of test tubes. About 0.1 and 0.2ml of the sample was pipetted out in two other test tubes.
- The volume was made upto 1ml using distilled water in all the test tubes. A tube with 1ml of buffer served as the blank.
- About 5ml of reagent C was added to each tube including the blank. Mixed well and allowed to stand for 10 minutes.
- To this, 0.5ml of reagent D was added, mixed well and incubated in dark for 30 minutes. Blue colour developed was read at 660nm.
- A standard graph was drawn and the amount of protein present in the sample was calculated.

Calculation

The amount of protein present in the sample was expressed in $\text{mg/gm} = \text{mg of protein} / \text{volume of test sample} \times \text{concentration of the standard}$.

Estimation of carbohydrate content (Hedge and Hofreiter, 1962)

Anthrone Method

Principle

Concentrated sulphuric acid hydrolyses the glycoside bond of carbohydrate to the given monosaccharides which were then dehydrated to furfural. The furfural reacted with anthrone (10 keto 9, 10-dihydroanthracene) to give the blue coloured complex which was measured calorimetrically at 630 nm.

Materials required

- 2.5 N HCl
- Anthrone reagent was prepared by dissolving 200mg anthrone in 100ml of ice-cold 95% H₂SO₄ Prepared freshly before use.
- Stock standard: 100mg of glucose was dissolved in 100ml of water.
- Working standard: 5ml of stock standard solution was diluted to 100ml using distilled water (50mg/ml)

Procedure

- About 100mg of the sample was taken in a boiling tube and was hydrolysed by keeping it in boiling water bath for three hours with 5ml of 2.5N HCl and cooled at room temperature .
- Then, it was neutralized with solid sodium carbonate until the effervescence ceases.
- The volume was made upto 100ml and centrifuged.
- The supernatant was collected and 0.5 and 1ml aliquot were taken for analysis.
- The standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard and a blank was maintained.
- The volume was made upto 1ml in all the tube including the sample tube by adding distilled water.

- Then 4ml of anthrone reagent was added and heated for eight minutes in a boiling water bath. Then, it was cooled rapidly and blue green colour developed was read at 630nm.
- A standard graph was drawn by plotting concentration of the standard on the x-axis versus absorbance on the Y-axis.
- From the graph, the amount of carbohydrate present in the sample was calculated.

Calculation

Amount of carbohydrate present in 100mg of sample

$$= \text{mg of glucose} / \text{volume of test sample} \times 100$$

STATISTICAL ANALYSIS

The data obtained from various biochemical observations were subjected to statistical analysis as per the procedure of Panse and Sukhatme (1978).

Preparation of leaf powder

The leaves of the two medicinal plants taken for the present study were collected, cleaned and air dried under shade for about three weeks. After drying, the leaves were then blended using a household electric blender. This fine powder was used for phytochemical analysis.



Plate 3 - Leaf powder of *Chromolaena odorata*



Plate 4 – Leaf powder of *Adhatoda vasica*

Preliminary phytochemical analysis

The leaf powders of both the plants were dissolved in various solvents and the preliminary phytochemical tests were carried out using the method of Harborne (1984).

Test for alkaloids

Mayer's Test

To 1ml of extract, 2ml of Mayer's reagent was added. Appearance of dull white precipitate indicates the presence of alkaloids.

Test for flavonoids

To one ml of extract, 1ml of neutral ferric chloride was added. The formation of brown colour confirmed the presence of flavonoids.

Test for steroids

Liebermann-Burchard Test

The extracts were dissolved in 2ml of chloroform to which 10 drops of acetic acid and five drops of concentrated sulphuric acid were added and mixed. The change of red colour through blue to green indicated the presence of steroids.

Test for terpenoids

Salkowski Test

Five ml of extract was mixed with 2ml of chloroform and conc. H_2SO_4 (3ml) was carefully added to form a layer. The formation of a reddish brown colour indicates the presence of terpenoids.

Test for quinones

To 1ml of extract, a few drops of conc. HCl was added. A yellowish brown colour was observed that showed the presence of quinone.

Test for phenols

To 1ml of extract, lead acetate solution was added and the precipitate formation indicated the presence of phenolic compounds.

Test for starch

To 1ml of extract, a few drops of iodine solution was added. Any characteristic colour changes showed the presence of starch.

Test for anthocyanin

NaOH Test

A small amount of extract was treated with 2ml of NaOH and observed for the formation of blue colour.

Test for proteins

Ninhydrin Test (Acetone)

Ninhydrin was dissolved in acetone. The leaf extract was treated with ninhydrin and observed for the formation of purple colour.

Test for carbohydrates

Molisch's Test

Two drops of Molisch's reagent was added to an aqueous or hydrochloric acid solution of the extract and two ml of concentrated sulphuric acid was added by the side of the test tube. The formation of reddish violet ring at the junction of the liquids indicated the presence of carbohydrates.

Test for cellulose

To 1ml of extract, a few drops of iodine solution was added followed by a few drops of sulphuric acid. Formation of dark brown (or) red colour showed the presence of cellulose.

Test for Fixed oil and fat

To 1ml of extract, a few drops of Sudan III solution was added. A shining orange colour obtained, showed the presence of fixed oil and fat.

CHAPTER -IV

RESULTS AND DISCUSSION

The biochemical study and phytochemical screening conducted in *Chromolaena odorata* and *Adhatoda vasica* showed the following results:

BIOCHEMICAL ANALYSIS OF THE LEAVES OF *Chromolaena odorata* and *Adhatoda vasica*

The biochemical parameters were studied using fresh leaf samples.

- Chlorophyll
- Protein
- Carbohydrate

ESTIMATION OF CHLOROPHYLL “a”, CHLOROPHYLL “b” AND TOTAL CHLOROPHYLL CONTENT

Chlorophyll ‘a’, chlorophyll ‘b’, and total chlorophyll content were calculated for the two medicinal plants.

The chlorophyll ‘a’ content was estimated to be 0.182 ± 0.20 mg and 0.124 ± 0.10 mg in the two medicinal plants *Chromolaena odorata* and *Adhatoda vasica* respectively (Table 1 & Figure 1).

The chlorophyll ‘b’ content was estimated to be 0.108 ± 0.05 mg and 0.046 ± 0.03 mg in *Chromolaena odorata* and *Adhatoda vasica* respectively (Table 1 & Figure 1).

The total chlorophyll content was higher in *Chromolaena odorata* and the value was 0.290 ± 0.25 mg (Table 1). The other medicinal plant studied showed a total chlorophyll content of 0.170 ± 0.12 mg (Table 1 & Figure 1). Chlorophyll ‘a’, ‘b’ and total chlorophyll content were found to be significantly higher in *Chromolaena odorata* when compared to *Adhatoda vasica*.

Among the biochemical parameters studied for the two medicinal plants viz., *Adhatoda vasica* and *Chromolaena odorata* belonging to the families Acanthaceae and

Asteraceae respectively, the protein content was significantly higher in *Adhatoda vasica*. Carbohydrate content, Chlorophyll 'a', Chlorophyll 'b' and total chlorophyll contents were significantly higher in *Chromolaena odorata*.

Table -1

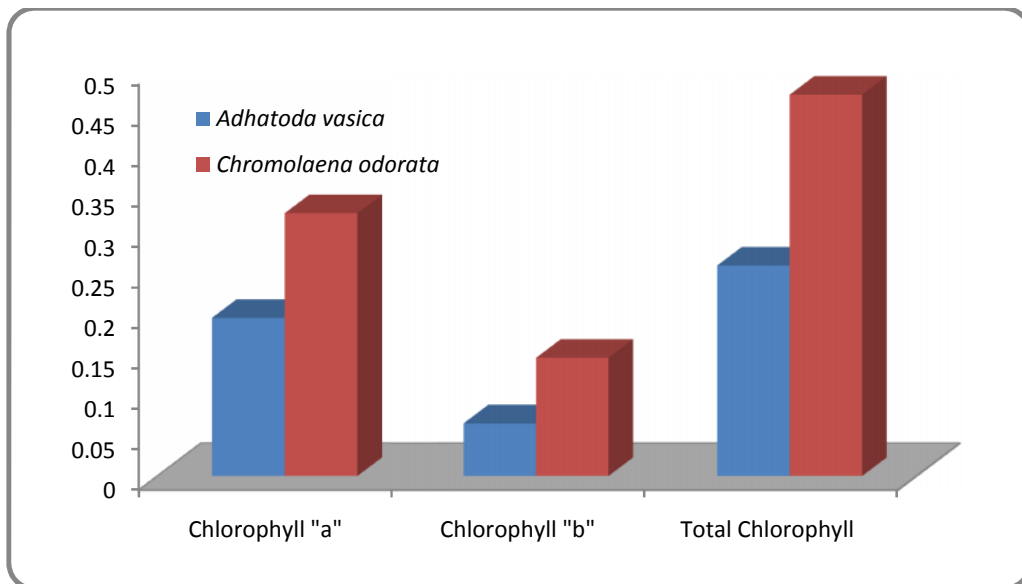
**Chlorophyll 'a', Chlorophyll 'b' and Total Chlorophyll contents
of the two medicinal plants**

Chlorophyll contents (mg /gm of leaf sample)	Medicinal plants	
	<i>Adhatoda vasica</i>	<i>Chromolaena odorata</i>
Chlorophyll a	0.124 ± 0.10	0.182 ± 0.20
Chlorophyll b	0.046 ± 0.03	0.108 ± 0.05
Total Chlorophyll	0.170 ± 0.12	0.290 ± 0.25

Values are mean ± SD of triplicates

Figure – 1

**Chlorophyll 'a', Chlorophyll 'b' and Total Chlorophyll contents of the
two medicinal plants**



ESTIMATION OF PROTIEN

The protein content of the two medicinal plants were observed and presented in Table 2 & Figure 2.

The highest protein content was estimated in *Adhatoda vasica* (21.4 ± 0.84 mg). *Chromolaena odorata* showed a protein content of 19.6 ± 1.414 mg (Table 2).

According to Bhumi *et al.* (2014), the biochemical studies on *Abrus precatorius* leaves, showed relatively high level of protein, which are the primary components of living organisms. Proteins are essential to maintain the structure and function of all life and vital for growth and development. The presence of higher protein level in the plants points towards their possible increase in food value or that a protein based bioactive compound could also be isolated in future.

According to Sunitha singh *et al.* (2012), leaf extracts of *Adhatoda* were analyzed for their biochemical composition. Result of their work indicated the presence of higher concentration of glycoside, saponin, proteins and amino acids.

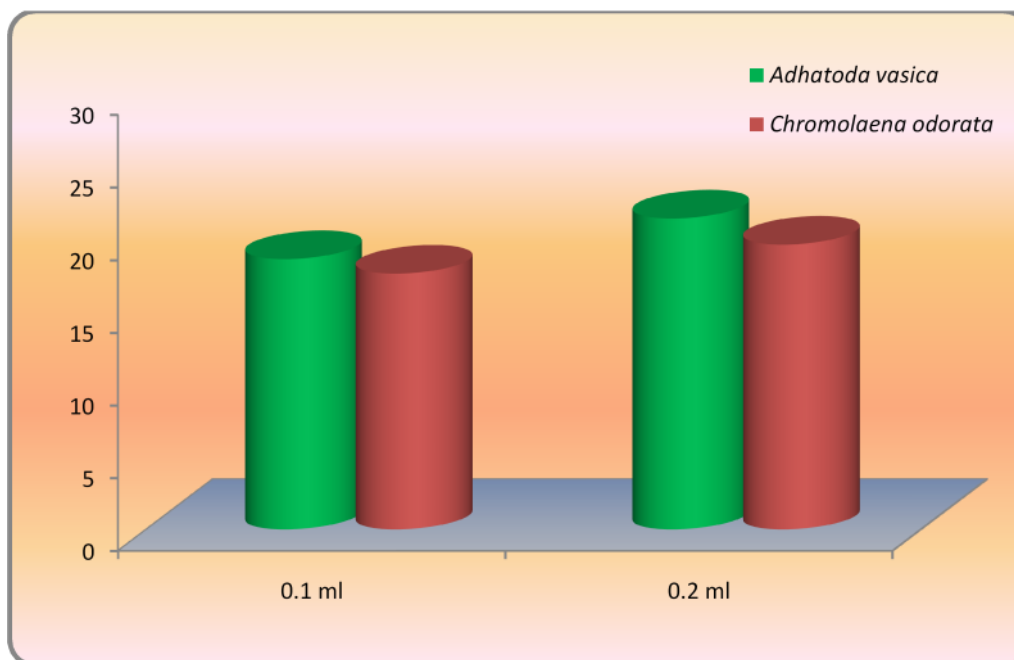
Manish Tadhani *et al.* (2008) have carried out biochemical studies in *Stevia rebundiana* leaves that showed relatively high content of protein, carbohydrate, potassium, calcium, magnesium, phosphorus and some active chemical compounds.

Table - 2
Protein content of the two medicinal plants

Plant	Protein(mg/gm of leaf sample)	
	0.1 ml	0.2 ml
<i>Adhatoda vasica</i>	18.6 ± 0.49	21.4 ± 0.84
<i>Chromolaena odorata</i>	17.6 ± 5.09	19.6 ± 1.414

Values are mean ± SD of triplicates

Figure 2
Protein content of the two medicinal plants



ESTIMATION OF CARBOHYDRATE

Among the medicinal plants taken for the study, the carbohydrate content was estimated to be higher in *Chromolaena odorata* (2.53 ± 0.11 mg) and the lower value was observed in *Adhatoda vasica* (1.57 ± 0.15 mg) (Table-3). Watal *et al.* (2014) have evaluated the presence of carbohydrates, glycosides and coumarins in the plant parts that are known to exert a beneficial action on immune system by increasing the body strength and hence are valuable as dietary supplements.

Shinwari *et al.* (2009) have undertaken biochemical studies in *Fumaria officinalis* and has revealed higher to moderate values of ash, fat, carbohydrates and protein contents compared to other species used in the herbal formulations.

Table - 3

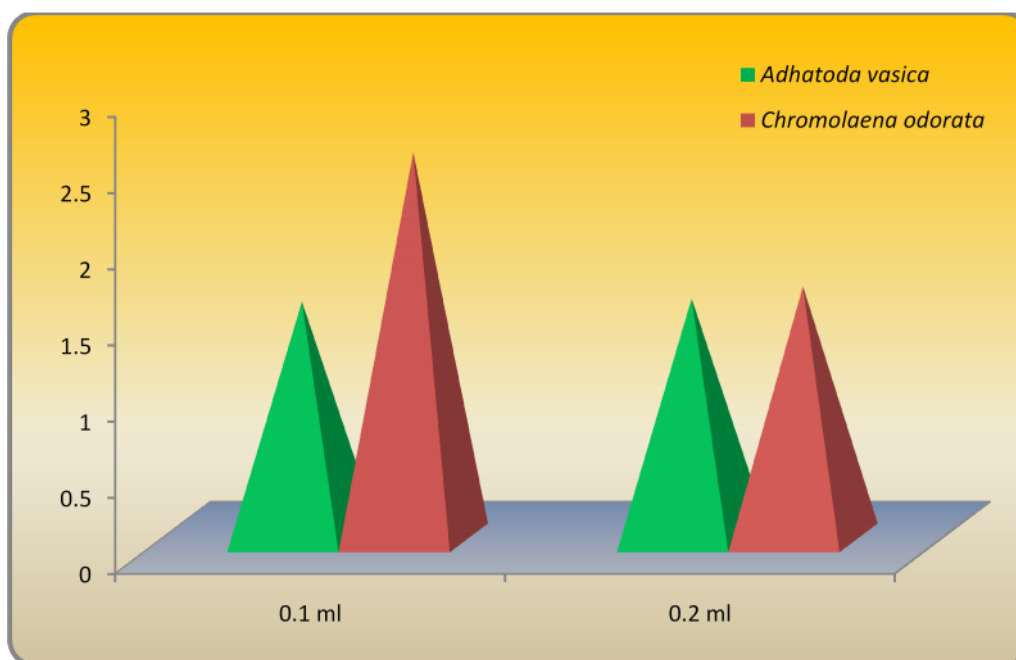
Carbohydrate content of the two medicinal plants

Plant	Carbohydrate (mg/gm of leaf sample)	
	0.1 ml	0.2 ml
<i>Adhatoda vasica</i>	1.55 ± 0.14	1.57 ± 0.15
<i>Chromolaena odorata</i>	2.53 ± 0.11	1.65 ± 0.21

Values are mean ± SD of triplicates

Figure -3

Carbohydrate content of the two medicinal plants



PRELIMINARY PHYTOCHEMICAL SCREENING OF LEAVES OF *CHROMOLAENA ODORATA* AND *ADHATODA VASICA*

In the present study using dry leaf powder, a preliminary phytochemical screening was carried out to identify the active constituents such as alkaloids, flavonoids, sterols, terpenoids, quinone, oil and fat, phenol, starch, anthocyanin, protein, carbohydrate and cellulose present in the leaves of the two medicinal plants (Table 4 & 5)

Preliminary phytochemical studies were done using different solvent extracts viz., ethanol, chloroform, petroleum ether, benzene and water for both *Adhatoda vasica* and *Chromolaena odorata*. The study showed the following results.

The dried and powdered leaves of *Adhatoda vasica* and *Chromolaena odorata* were dissolved in different solvents viz., water, ethanol, chloroform, petroleum ether, benzene and the extracts thus obtained were analyzed for the presence or absence of secondary metabolites.

In *Chromolaena odorata*, flavonoids, starch, carbohydrate, cellulose, oil and fat were observed in all the five solvent extracts. Alkaloids were completely absent in all the five solvent extracts of leaves of *Chromolaena odorata*. Protein was observed only in chloroform and benzene extracts. Except for water, phenol content was observed in the other four solvents extracts of *Chromolaena odorata*. Among the five solvent extracts of the leaves of *Chromolaena odorata*, steroids was observed in chloroform, ethanol and benzene, but, terpenoids was seen in water, chloroform and petroleum ether extracts. Quinone was present in water and petroleum ether extracts. Anthocyanin were present only in ethanol and petroleum ether extracts of *Chromolaena odorata*. (Table 4)

Phytochemical screening of various solvent extracts of *Taraxacum officinale* indicated the presence of saponins, flavonoids, flavons, flavonols, chalcones, phlobatanins and cardiac glycosoides, while alkaloids, tannins, anthraquinones, coumarins, steroids and terpenoids were absent (Mir *et al.*, 2013)

Phytochemical screening of leaves of five medicinal plants i.e. *Phyllanthus amarus*, *Clerodendrum viscosum*, *Ailanthus exelsa*, *Syzigium cumini* and *Cassia occidentalis* using various solvent extracts revealed the presence of steroids, saponin,

alkaloids, flavonoids, glycosides, phenolic compounds, tannin, terpenoids and lignin (Joseph *et al.*, 2012).

Ketaren Bunga Raya (2015) reported that, young plant parts contain higher amount of phytochemicals, ascorbic acid and chlorophyll compared with matured parts confirming that phytochemical content of *Clinacanthus nutans* decreases when plants tend to maturity.

The preliminary phytochemical analysis of *Chromolaena odorata* extracts revealed the presence of alkaloids, glycosides, flavonoids, saponins and tannins (Alisi *et al.*, 2008).

The phytochemical analysis showed the presence of phenols, tannins, alkaloids, anthraquinone, saponins, flavonoids and reducing sugars in the leaves of *Justicia adhatoda* (Pathak, 1970).

Table 4**Preliminary phytochemical analysis of *Chromolaena odorata***

Phytochemicals	Water	Chloroform	Ethanol	Petroleum ether	Benzene
Alkaloids	-	-	-	-	-
Flavonoids	+	+	+	+	+
Steroids	-	+	+	-	+
Terpenoids	+	+	-	+	-
Quinone	+	-	-	+	-
Phenols	-	+	+	+	+
Starch	+	+	+	+	+
Anthocyanin	-	-	+	+	-
Protien	-	+	-	-	+
Carbohydrate	+	+	+	+	+
Cellulose	+	+	+	+	+
Oil and fat	+	+	+	+	+

‘+’ - Present.

‘-’ - Absent

In *Adhatoda vasica*, among the five solvents used for extraction, petroleum ether and benzene showed the presence of alkaloids. Cellulose, flavonoid, starch, oil and fat were found in all the five solvent extracts of the leaves of *Adhatoda vasica*. (Table 5) Anthocyanin was observed in chloroform, ethanol and benzene extracts, but, steroid was present in four solvent extracts, except petroleum ether. Carbohydrate was present in four solvent extracts except water. Protein was observed in water, chloroform and benzene extracts, whereas, out of the five solvent extracts, water, ethanol and petroleum ether showed the presence of terpenoids. Phenol was present in four of the five solvent extracts, except water.

A phytochemical test carried out by Vikrant Arya *et al.* (2012) on the leaf extracts of *Psidium* revealed the presence of various phytoconstituents like flavonoids, tannins, triterpenoids, saponins, sterols, alkaloids and carbohydrates.

According to Adenike *et al.* (2010), the phytochemical screening of three spices such as – *Allium sativum* L., *Zingiber officinale* Rosc. and *Capsicum frutescens* L. revealed the presence of rich phytonutrients including alkaloid, tannin, carotenoids, saponin and flavonoids.

Table 5**Preliminary phytochemical analysis of *Adhatoda vasica***

Phytochemicals	Water	Chloroform	Ethanol	Petroleum ether	Benzene
Alkaloids	-	-	-	+	+
Flavonoids	+	+	+	+	+
Steroids	+	+	+	-	+
Terpenoids	+	-	+	+	-
Quinone	-	+	-	+	+
Phenols	-	+	+	+	+
Starch	+	+	+	+	+
Anthocyanin	-	+	+	-	+
Protien	+	+	-	-	+
Carbohydrate	-	+	+	+	+
Cellulose	+	+	+	+	+
Oil and fat	+	+	+	+	+

‘+’ - Present.

‘-’ - Absent

Plants have played a critical role in maintaining human health and civilizing the quality of human life for thousands of years. Medicinal plants have bioactive compounds which are used for curing various human diseases. Chlorophyll, proteins and common sugars play an important role in healing. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of new drugs (Wadood *et al.*, 2013).

CHAPTER –V

SUMMARY AND CONCLUSION

The result of the present study revealed that the leaves of the two medicinal plants viz., *Chromolaena odorata* and *Adhatoda vasica* contain the important bioactive compounds that can play a crucial role in providing nutritional significance and health effects in man.

The two medicinal plants taken for the present study possess medicinal property. The biochemical parameters studied showed significantly higher chlorophyll 'a', chlorophyll 'b', total chlorophyll and carbohydrate content in *Chromolaena odorata*. The protein content was found to be significantly higher in *Adhatoda vasica*.

The presence of phytoconstituents make the plant useful for treating different ailments and have a potential of providing useful drugs of human use. In the present study, we have found that most of the biologically active phytochemicals were present in the ethanolic and aqueous extracts of leaves of *Chromolaena odorata*. Since the ethanolic extract contains more constituents, it can be considered beneficial for further investigation.

The medicinal values of plants i.e., their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds produce a definite physiological action on human body. Flavonoids are reported to possess many useful properties including, anti-inflammatory, antimicrobial, enzyme inhibition, oestrogenic, antiallergic, antioxidant and anti-tumour activity. Flavonoids are a group of naturally occurring phenolic compounds primarily from fruits and vegetables.

The presence of phytochemical like flavonoids in the leaves of medicinal plants indicate their medicinal action encountered in its therapeutic uses. All the plants studied possess important biochemical compounds and secondary metabolites which provide great contributions to our health management.

The medicinal plants are rich in phytochemicals (secondary metabolites) and essential oils of therapeutic importance. Development of novel efficacious drugs or drug combinations for the treatment of helminthic infections is of high research priority.

The quantitative analysis of the various bioactive compounds present in the two medicinal plants could significantly contribute to maintain and promote healthy life for human and animals.

REFERENCES

- Abdul Wadood, Mehreen Ghufuran, Syed Babar Jamal, Muhammad Naeem, Ajmal Khan, Rukhsana Ghaffar and Asnad (2013). Phytochemical Analysis of medicinal plants occurring in Local Area of Mardan. *Biochem. Anal. Biochem.* **2**: 4.
- Adnan, M., Hussain J., Shah M.T., Ullah F., Shinwari J.K., Bahadar A., Khan A.L. (2010). Proximate and nutrient Composition of Medicinal Plants of Humid and Sub-humid regions in Northwest Pakistan. *J. Med. Plant. Res.* **4**: 339-345.
- Adriana, B., Adriana, A.B.A., Tatiana C.P., Terezinh, J.A.P and Myrn, S. (2006). Occurance of toxigenic fungi in herbal drugs. *Braz. J. Micro.* **37(1)**: 1-4.
- Afolayan, A. J. (2003). Extracts from the shoots of *Arctotis artotoides* inhibit the growth of bacteria and fungi. *Pharm. Biol.* **41**: 22-25.
- Ajaykumar Gautam and Shubhi Avasthi (2013). *Collectotrichum gleosporioides* on *Adhatoda vasica* in India. *J. Agric. technol.* **9(3)**: 581-587.
- Akomas, S.C and Ijioma, S.C. (2014). Bleeding and clotting time effect of ethanolic extracts of *Chromolaena odorata* versus *ocimum gratissimum* treated albino rats. *Comp.J. Med. Sci.* **2(1)**: 9 -13.
- Alam, M.T., Karim, M.M., Shakila, K.N. (2009). Antibacterial activity of different organic extracts of *Achyranthes aspera* and *Cassia alata*. *J. Sci. Res.* **1**: 393-398.
- Alisi, C. S. and Onyeze, G. O. C. (2008). Nitric oxide scavenging ability of ethyl acetate fraction of methanolic leaf extracts of *Chromolaena odorata*. *Afri. J. Biochem. Res.* **2(7)**: 145-150.
- Alisi, C.S., G.O.C. Onyeze, O.A., Ojiako, and C.G. Osuagwu. (2011). Evaluation of the Protective Potential of *Chromolaena odorata* Linn. Extract on Carbon Tetrachloride Induced Oxidative Liver Damage. *Int. J. Biochem. Res. Rev.* **1(3)**: 69-81.

- Amazu, L.U., Omoregie, P., Ajugwo A.O., I.fezulike C.C and Azikiwe. C.A. (2014). Anticunvulsant potency of the leaves extract of *chromolaena odorata* in rats. *Unique. Res. J.* **1(6)**: 064 -069.
- Amin Mir, M., Sawhney, S.S., Jassal, M.M.S. (2013). Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. *J. Pharm. Pharm.* **2(1)**: 001 – 005.
- Anubha Arora, (2013). Phytochemical analysis of methanolic extracts of leaves of some medicinal plants, *Biol. form –An int. J.* **5(2)**: 91-93.
- Anyasor, G.N., Aina, D.A., Olushola, M. and Aniyikaye, A.F. (2011). Phytochemical constituent, proximate analysis, antioxidant, antibacterial and wound healing properties of leaf extract of *Chromolaena Odorata*. *Anal .Biol. Res.* **2(2)**: 441-451.
- Aravind Dangi, Shivkanth patel, Yadhuvanshi. P.S. (2015). Phytochemical screening and Assessment of *Adhatoda vasica* (Leaf) for anti asthmatic activity. *Panacea. J. pharm. pharm. sci.* **4(3)**: 680-704.
- Arun kumar, S., Muthuselvam, M and Rajasekaran, R. (2010). Analysis of Phytochemical constituents and antimicrobial activity of some Southern Indian medicinal plants. *J. Pharm.Res.* **3(8)**: 1841-1843.
- Ashvin Godghate and Rajaram sawant, (2013). Qualitative phytochemical analysis of chloroform extract of leaves of *adhatoda vasica* Nees. *Rasayan. J.chem.* **6(2)**: 107-110.
- Asolkar, L.V., Kakkar, K.K., Chakra O.J. (1992). Second Supplement to Glossary of Indian Medicinal Plants with active principles. *Part I Publication and information Directorate (CSIR), New Delhi, India.* **Pp**: 78-84.

- Atal, C.K. (1980). Chemistry and Pharmacology of vasicine: A new Oxytocin and abortifacient. *Indian Drugs*. **15**: 15-18.
- Badami, S., Aneesh, R., Sankar, S., Sathishkumar, M.N., Suresh, B., Rajan, S. (2003). Antifertility activity of *Derris brevipes* variety coriacea. *J. Ethnopharmacol.* **84**: 99-104.
- Bamba, D., J.M. Bessiere, L. Marion, Y. Pelissier, and I. Fouraste. (1993). “Essential Oil of *Eupatorium odoratum*”. *Planta Medica*. **59**: 184-185.
- Baquar, S.R. (1997). Medicinal and Poisonous Plants of Pak. *J. Medicine Karachi*. **Pp** : 95-96.
- Bedi, G., Tonzibo, Z.F., Chopard C., Mahy J.P., N. guessan. T.Y. (2004). Etudes effets antidouleurs des huiles essentielles de *Chromolaena odorata* et de *Mikania cordata*, par action sur la Lipoxygenase L-1 de soja. *Phys. Chem. News*. **15**: 124 –127.
- Bhat, V.S., Nasavat, D.D., Mardikar, B.R. (1978). *Adhatoda vasica*- an Ayurvedic plant. *Indian Drugs*. **15**: 62-66.
- Bhumi .G. and Savithramma. (2014). screening of pivotal medicinal plants for qualitative and quantitative phytochemical constituents. *Int. J. pharm.pharm. Sci.* **6(3)**: 63-65.
- Bhumi, G., Ratna Raju, Y., Savithramma, N. (2014). Screening of zinc oxide nanoparticles for cell proliferation synthesized through *Adhatoda vasica* Nees. *Int. J. Drug. Dev. & Res.* **6(2)** : 0975 – 9344.
- Biller, A., Boppre, M., Witte, L. and Hartmann, T. (1994). Pyrrolizidine alkaloid in *Chromolaena odorata*. Chemical and chemo ecological aspects. *Phyto.chem.* **35**: 615–619.
- Bruneton, J. (1995). Pharmacognosy, Phytochemistry of Medicinal Plants. *Lavoisier, France* pp: 265–380.

- Che Man, N.B. (2010). Phytochemical analysis of the leaves of *Chromolaena odorata* (Asteraceae). BSc Hons. Thesis, Faculty of Applied Sciences- University of Technology. *Mara*: 9-12.
- Claeson, U.P., Malmfors, T., Wikman, G., Bruhn, J.G. (2000). *Adhatoda vasica*: a critical review of ethno pharmacological and toxicological data. *J. Ethnopharmacol.* **72**: 1-20.
- Cordell, G.A. (1993). Pharmacognosy. New roots for an old science. In: Atta-ur-Rahman, Basha F.Z., editors. Studies in natural product chemistry. Bioactive natural products (Part A). *Elsevier.* **13**.
- Cui, S., S., Tan, G. Ouyang,S., Jiang, and J. Pawliszyn. (2009). “Headspace Solid-Phase Micro extraction Gas Chromatography-Mass Spectrometry Analysis of *Eupatorium odoratum* Extract as an Oviposition Repellent”. *J.Chrom. B, Anal.technol. Biomed. Life Sci.* **877 (20-21)**: 1901–1906.
- Das, N., Islam, M.E., Jahan, N., Islam, M.S., Khan, A., Islam, M.R. and Parvin, M.S. (2014). Antioxidant activities of ethanol extracts and fractions of *Crescentia cujete* leaves and stem bark and the involvement of phenolic compounds. *BM, Compl. Altern. Med.* **14**: 45.
- Debashisha Panda, Santosh Kumar Dash, Gouri Kumar Dash.(2010). Qualitative phytochemical analysis & investigation of antihelmintic and wound healing potentials of various extracts of *chromolaena odorata linn*. Collected from the locality of mohuda village, berhampur (south orissa). *Int. J. Pharm.Sci. Rev. Res.* **1(2)**: 122-126.
- Dey, S.K., De A., Karmarker, S., Dep, K., Charkarborty, S., Samanta, A and Mukherjee, A. (2009). Ethanobotanical study in a remote district of West Bengal, India. *Pharmabit* xx (2): 91-96.

- Dinesh Mishra and Arti Broker. (2009). An Ethno medicinal Study among the Gond and Chhattisgarh: India. *Antrocom*. **5(1)**: 61-65.
- Dogra, K. S., Kohli, R. K. and Sood, S. K. (2008). Impact of some Invasive Species on the Structure and Composition of Natural Vegetation of Himachal Pradesh, India. *Int. J. Biodiv. Conserv.* **3(2)**: 008-015.
- Egunjobi, j.k. (1969). Some common weeds of western Nigeria. *Bull. Res. Div. Min. Agr. Nat. Resources, Western state Nigeria*.
- Fukamiya, N., Lee, K. (1986). Antitumor agents, 81. Justicidin-A and diphyllin, two cytotoxic principles from *Justicia procumbens*. *J. Nat. Prod.* **49**: 348-350.
- Gill, L.S. (1992). Ethano medical uses of plants in Nigeria. *University of Benin Press, Benin City Nigeria*: 10-30.
- Gloria A. Otunola, Oyelola B. Oloyede Adenike T. Oladiji and Anthony J. Afolayan (2010). Comparative analysis of the chemical composition of three spices – *Allium sativum* L. *Zingiber officinale* Rosc. and *Capsicum frutescens* L. commonly consumed in Nigeria. *Afri. J. Biotechnol.* 9(41): 6927-6931.
- Gupta, M., Mazumdar, U.K., Kumar, T.S., Kumar R.S. (2004). *Iran. j. pharm. ther.* **3(1)**: 12-20.
- Gupta, R., Thakur, B., Singh, P., Singh, H.B., Sharma, V.D., Katoch, V.M. (2010). Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates. *Indian J. Med. Res.* **131**: 809-813.
- Harborne, J.B. (1984). Phytochemical methods. II.Ed. *In Chapman and Hall, New York*. pp. 21-26.
- Hedge, J.E. and Hofreiter, B.T. (1952). Determination of total carbohydrate by anthrone method. In: *Carbohydrate chemistry (Eds.), Whistler, R.L. and Bo Miller, J.N., Academic Press, New York* .pp.17.

- Hill, A.F. (1952). *Economic Botany. A textbook of useful plants and plant products*. 2 nd edn. *McGraw-Hill Book Company Inc, New York*.
- Holm, L. G., Plucknett, D. L., Pancho, J. V. and Herberger, P. D. (1977). *The World's Worst Weeds: Distribution and Biology*. Honolulu, HI: *University Press of Hawaii*.
- Hui, H.Y., Chang, C.J., McLaughlin, J.L., Powell, G.P. (1986). Justicidin B, a bioactive trace lignan from the seeds of *Sesbania drummondii*. *J.Nat .Prod* .**49**: 1175-1176.
- Hung, T. M., Cuong, T.D., Dang, N. H., Zhu, S., Long, p.q., komatsu, k. and Min B. S. (2011). Flavonoid Glycosides from *Chromolaena odorata* leaves and Their in Vitro Cytotoxic Activity. *Chem. Pharm. Bull.* **59(1)**: 129-131.
- Ibegbulam, C.M., Anyakwo, C.N., Ukoba, O.K., Ekwujuru, R.I., Ekos, E. and Nwaosu, C.S. (2011). Evaluation of inhibitive property of *Chromolaena odorata* extract on aluminum in 1MHCl and 0.5M NaOH environment. *Pac .J. sci. technol.* **12(2)**: 31-38.
- Igboh M.N., Ikewuchi C.J., Ikewuchi C.C. (2009). Chemical Profile of *Chromolaena odorata* L. (King and Robinson) Leaves. *Pak. J. Nutr.* **8(5)**: 521–524.
- Ignacimuthu, S and Shanmugam, N. (2010). Antimycobacterial activity of two natural alkaloids, vasicine acetate and 2-acetyl benzylamine, isolated from Indian shrub *Adhatoda vasica* Nees. Leaves. *J. Bio. sci.* **35(4)**: 565–570.
- Inderjit Kaur, P.K., Chauhan., Jaryall., Shubham Saxena and Kanishak. (2012). Antioxidant and antimicrobial activity of leaf extract of *adhatoda vasica* against the bacteria isolated from the sputum samples of asthmatic patients. *Int. J. Drug. Res. Tech.***2 (3)**: 273-278.
- Iwu, M.M., A.R., Duncan and C.O. Okunji, (1999). New antimicrobial of plant origin. Reprinted from: *Perspective on new crops and new uses*. *J. Janick Edn., Alexandria, V.A: ASHS Press*.

- Jain, S.K. and Defilipps, R. A. (1991). Medicinal Plants of India. *Reference Publications, Michigan, USA*. **1**:1-92.
- Javid Hussain, Abdul Latif Khan, Najeeb ur Rehman, Muhammad Hamayun, Zabta Khan, Shinwari, Wasi Ullah and In-Jung Lee. (2009). Assessment of herbal products and their composite medicinal plants through proximate and micro nutrients analyses. *J. Medi.Plants. Res.* **3(12)** : 1072-1077.
- Jayakar, B., and Suresh, B. (2003). Anti hyperglycemic and hypoglycemic effect of *Aporosa lindleyana* in normal and alloxan induced diabetic rats. *Ethnopharmacol.* **84(2-3)**: 247-249.
- Jayapriya, G and F.Gricilda shoba. (2015). Phytochemical analysis, Antimicrobial Efficiency and determination of Bioactive Components from leaves of *Justicia adhatoda*. *Asian J. Plant .Sci. Res.* **5(2)**: 43-51.
- Jeya S., Veerakumari, L. (2013). Effect of *Adhatoda vasica* and *Piper Betle* Leaves Extracts on Motility and Acetyl cholinesterase of *Cotylophoron Cotylophorum Invitro*. *Int. J.Sci. Res.* **4(6)** : 2319 -7064.
- Jha A.K., Kumar V., Prasad K. (2011). Biosynthesis of metal and oxide nano particles using Orange juice. *J. Bio .nano. Sci.* **5(2)**: 162-166.
- Joseph, B.S., Kumbhare, P.H., Kale, M.C.(2013). Preliminary phytochemical screening of selected Medicinal plants. *Int. Res. J. Sci. & Eng.* **1(2)**: 55-62.
- Joshi, A.R. and Joshi, K.(2000). Indigenous knowledge and uses of medicinal plants by local communities of the Kali Gandaki Watershed Area, Nepal. *J. Ethnopharmacol.* **73**: 175-183.
- Karthikeyan, A., Shanti, V and Nagasathya, A. (2009). Preliminary Phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda vasica* (L). *Int. J. green Pharm.* **3**: 78-80.

- Kasamota, I.T., Nakabayasi, T., Kida, H. (1995). Screening of various plant extracts used in Ayurvedic medicine for inhibitory effects on human immuno deficiency virus type I (HIV- protease). *Phytother. Res.* **9**: 180-184.
- Kaushik, P., Kaushik,D., Khokra,S and Choudhary.B. (2009). Abutilonindicum Atibala: Ethno- botany, Phytochemistry and Pharmacology. *Int. J. Pharm. Clin. Res.* **1(1)**: 49.
- KavithaVijayaraghavan, S., Mohamed Ali, R., Maruthi. (2013).Studies on Phytochemical Screening and Antioxidant Activity of *Chromolaena Odorata* and *Annona squamosa*. *Int. J. Innov. Res. Sci. Engi. technol.* **2**: 7315-7321.
- Kedare, S.B. and Singh, R.P. (2011). Genesis and development of DPPH method of antioxidant assay. *J. Food. Sci. Technol.* **48(4)**: 412–422.
- Ketaren Bunga Raya, Siti Hajar Ahmad, Sanusi Fatin Farhana, Munirah Mohammad, Nor Elliza Tajidin, Anwar Parvez.(2014). Changes in phytochemical contents in different parts of *Clinacanthus nutans* (Burm. f.) lindau due to storage duration. *Bragantia, Campinas.Universiti Putra Malaysia – UPM, Department of Crop Science, Seri Kembangan, 43400, Selangor, Malaysia.*
- Kiran kumari, S.P., Satya vani.Y. Sridevi, V., Chandana Lakshmi, M.V.V. (2012). Separation and observation of plant pigments in fertilizers effected medicinal plants using paper chromatography. *Int. J. engi. Sci & adv. technol.* **2(2)**: 317 – 326.
- Kpoviessi, S., Gbaguidi, F., Gbenou, J., Accrombessi, G., Haddad,M., Moudachirou, M., Quetin-Leclercq, J. (2006). Allelopathic effects on cowpea (*Vigna unguiculata* (L.) Walp) plant and cytotoxic activities of sterols and triterpenes isolated from *Justicia anselliana* (NEES) T. Anders. *Electron .J .Nat .Subs.* **1**: 12-19.
- Kumar, A., Ram, J., Samarth, R.M., Kumar, M. (2005). Modulatory influence of *adhatoda vasica* Nees leaf extract against gamma irradiation in Swiss albino mice. *Phyto.med.* **12**: 285-293.

- Lady Jane G. Morilla, Nanette Hope N., Sumaya, Henry I. Rivero and Ma. Reina Suzette B., Madamba. (2014). Medicinal Plants of the Subanens in Dumingag, Zamboanga del Sur, Philippines. *International Conference on Food, Biological and Medical Sciences (FBMS) Jan. 28-29, Bangkok (Thailand)* **pp** : 38-43.
- Lovet, T., kigigha, and douye victor zige. (2013). Activity of *chromolaena odorata* on enteric and superficial etiologic bacterial agents. *Ajrc .J.* **1(11)**: 266.
- Lowry, O.H., Rosen rough, N.S., Farr, A.L. and Randall, R.J. (1951). Protein measurement with folin phenol reagent. *J. Biol .Chem.* **193**: 267-273.
- Mahajan, S.K. (2007). Traditional herbal remedies among the tribes of Bijagarh of West Nimar District, Madhya Pradesh. *Indian. J. Tradit. Know.* **6(2)**: 375-377.
- Mahbubur Rahman A.H.M., Kabir R.Z.M.F., Rafiul Islam A.K.M and Zaman A.T.M.N. (2013). Medico-Botanical Investigation by the Tribal People of Naogaon District, Bangladesh. *J. Med. Plants. Studies.* **1(4)**: 136-147.
- Manasi Dash, Jayanta kumar patra, prasanna priyadarshni panda. (2008). Phytochemical and antimicrobial screening of extracts of *Aquilaria agallocha.Roxb.* *Afr.J.Biotechnol.* **7**: 353 – 357.
- Manish Tadhani and Rema Subash. (2006). Preliminary Studies on *Stevia rebaudiana* Leaves: Proximal composition, Mineral Analysis and Phytochemical screening. *J.med. sci.* **6(3)**: 321-326.
- Manjulika yadav, Sanjukta chatterji, sharad kumar gupta, geeta watal. (2014). Preliminary phytochemical screening of six medicinal plants used in traditional medicine. *Int. J.Pharm. Pharm. Sci.* **6(5)**: 539-542.
- Manjunath, B.L. (1948), “The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products”. *Counc. Sci. Ind. Res. Delhi.* **pp**: 31-38.

- Matthew, Masoli, Denise, Fabian, Shaun, Holt and Richard, Beasley (2002), "Medical Research Institute of New Zealand Wellington". *New-Zealand University of Southampton, United Kingdom*. **2(11)**: 486-490.
- Metwally, A.M., Ekejuba, E.C (1981). Methoxylated flavonols and flavones from *Eupatorium odoratum* I. *Planta Medica*. **pp**: 402- 403.
- Mohammed, S. (2006). Anticancer agents from medicinal plants, Bangladesh. *J. Pharmacol.* **1** : 35-41.
- Mohanta, T.K., Patra, J.K., Rath, S.K., Pal, D.K., Thatoi, H.N. (2007). Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semicarpus anacardium*. *L.f. Sci. Res. Essay*. **2(11)**: 486-490.
- Murugesan, S., Pannerselvam, A., Tangavelou, A.C. (2011). Phytochemical screening and antimicrobial activity of the leaves of *Memecylo umbellatum* burm. F. *J.Appl.Pharm. Sci.* **1**: 42-45.
- Nadkarni,K.M (1976) Indian Materia Medica, With Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic & Home Remedies, *Appendices & Indexes*. *Popular Prakashan, Bombay*. **pp** : 40–43.
- Nandre, B.N., S. R. Bakliwal., B. R. Rane., S. P. Pawar. (2012). A review on *adhatoda vasica*. *Int. J. pharm. sci.* **3**: 3232- 3245.
- Narula,S., Shrvastava, P.S. and Rangaswamy. N.S. (2000). *In vitro* cultural studied on *Dioscorea* sp. *J. Trop. Med. Plants.* **1**: 60-74.
- Nyananyo, B.L. (2006). Plants from the Niger Delta. *Onyoma research publication. Port-Harcourt, Rivers State Nigeria* : 85.

- Panase ,V.G. and Sukhatme, P. V. (1978). Statistical methods for agricultural workers, 3rd Edn. ICAR, New Delhi, Pp: 347.
- Pandita K., Bhatia M.S., Thappa R.K., Agarwal S.G., Dhar K.L., Atal C.K. (1983). Seasonal variation of alkaloids of *Adhatoda vasica* and detection of glycosides and N-oxides of vasicine and vasicinone. *Planta Medica*. **48**: 81-82.
- Patel, V.K., Venkata-Krishna-Bhatt, H. (1984). In vitro study of antimicrobial activity of *Adhatoda vasica*. (L) (Leaf extract) on gingival inflammation, A preliminary report. *Indian .J. Med. Sci*. **38**: 70-72.
- Pathak, R.P. (1970). Therapeutic Guide to Ayurvedic Medicine (A Handbook on Ayurvedic Medicine). Shri Ramdayal Joshi Memorial Ayurvedic Research Institute. *Publ. Series No. 1*, **121**: 208–209.
- Phan, T.T., Hughes, M.A., Cherry G.W. (2001). Effects of an aqueous extract from the leaves of *Chromolaena odorata* Eupolin on the proliferation of human keratinocytes and on their migration in an in vitro model of re epithelialisation. *Wound Rep Reg*. **9**: 305–313.
- Pushpangadan,P., Nyman,U., George,V. (1995). Glimpses of Indian Ethno pharmacology. *Tropical Botanic Garden and Research Institute, Kerala*: 309-383.
- Rak, A.E., Ibrahim, N.A. and Bujang, M. (2012).Potential of *Chromolaena odorata* and indigenous bacterial consortium for oily waste water treatment. *J. Basic. Appl. Sci*. **8**: 196-201.
- Ramya, S and Jayakumararaj R. (2009). Anti feedant Activity of Selected Ethno-botanicals used by Tribals of Vattal Hills on *Helicoverpa armigera* (Hubner). *J. Pharm. Res*. **2(8)**: 1414-1418.
- Riaz Ullah, Iqbal Hussain, Jameel A. Khader, Naser M. AbdeIslam, Shabir Ahmad, Sadar jan, Kamin khan.(2013).Antibacterial and Essential oil Analysis of the Medicinal plant *adhatoda vasica* leaves. *Life. Sci. J*. **10(2)**: 787-790.

- Sachin B. Agawane, Vishakha Tiwari, Suraj K. Chavan and Santhosh S. Koratkar. (2015). *In vitro* and *in vivo* inhibition of post prandial Hyperglycemia (Type II Diabetes) by use of *Adhatoda vasica* in wistar Rats. *Int.J. pharm. Pharm. Res.* **4(4)**: 37-52.
- Sachin Kumar, Hotam Singh Choudhary, Chandrabhan Seniya. (2011). *In vitro* antibacterial study of aqueous and methanolic extracts of some selected medicinal plants. *J. Chem. Pharm. Res.* **3(4)**: 854-860.
- Sajise, P.E., Palis, R.K., Norcio, N.V., Lales, J.S. (1974). The biology of *C.odorata* L.King and Robinson.1. Flowering behaviour, pattern of growth and nitrate metabolism. *Phil. Weed Sci. Bull.* **1**: 17-24.
- Salalamp, P., Chuakul, W., Temsiririrkkul, R., Clayton,T. (1996). Medicinal plants in Thailand. *Amarin Printing and publishing Public Co, Bangkok*, **pp** :1-21.
- Sampath Kumar, K. P., Bhowmik Debjit, Chiranji B., Tiwari Pankaj, Kharel Rakesh. (2010). "Indian traditional herbs *Adhatoda vasica* and its medicinal application". *J.chem.Pharm.Res.* **2(1)**: 240 -245.
- Santhosh kumar singh, Jay Ram Patel, Prashant kumar Dubey, Aravind Dangi, (2014). Pharmacognostic study and phytochemical screening of leaf *adhatoda vasica*. *J. med. Plants. Studies.* **2(4)**: 29-41.
- Santoyo, S., Cavero, S., Jaime, L., Ibanez, E., Senorans, F.J. and Reglero, G.(2005). Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. essential oil obtained via supercritical fluid extraction. *J. Food. Prot.* **68**: 790-795.
- Seema, S., Sharma, D., Raghuvanshi, R.K., Sharma, R.N and Chandra ant P. (2010). Cytological studies Of *Adhatoda* L sp and *Barleria* sp. *The Bioscan.* **5(1)**: 67-70.
- Sharma, M.P., Ahmad, J., Hussain, A and Khan, S. (1992). Folklore medicinal plants of Mewat (Gurgaondistrict), Haryana, India. *Pharm. Biol.* **30**: 129-134.

- Sheetz M.J., King G.L. (2002). Molecular understanding of hyperglycemia's adverse effects for diabetic complications. *Jama*. **288 (20)**: 2579-88.
- Shetonde O. Mihigo, Ishmael B., Masesane, K., Sichilongo, Luvenga Ndombele. (2015). Preliminary GC-MS Profiling and Anti-bacterial activity Investigation of *Chromolaena odorata* Linn. R.M. King and H. Robinson (Asteraceae). *Int. J. Chem. Aqua. Sci.* 1(1): 38-49.
- Shinwari, Z. K. (2010). Medicinal Plants Research in Pakistan. *J. Med. Plant Res.* **4(3)**: 161-176.
- Siddiqui, M.B., Hussein, W. (1993). Traditional treatment of gonorrhoea herbal drugs in the province of central Uttar Pradesh, India. *Fitoterapia*. **64** : 399-403.
- Sofowora, L.A. (1993). Medicinal plants and traditional medicine in Africa. *Spectrum Books Ltd, Ibadan. Harborne. Pp*: 55-71.
- Sukanya,S.L., Sudisha,J., H.S. Prakash, H.S and Fathima, S.K. (2011). Isolation and characterization of antimicrobial compound from *Chromolaena odorata*. *J. Phytol.* **3(10)**: 26-32.
- Suksamran, A., Chotipong, A., Suavansri, T., Boongird, S., Timsuksai, P., Vimuttipong, S., & Chuaynugul, A. (2004). Anti mycobacterial activity and cytotoxicity of flavonoids from the flowers of *Chromolaena odorata*. *Arch. Pharm. Res.* **27(5)**: 507-511.
- Sunita Singh, Arshad Hussain and Dhananjay Singh. (2014). Phytochemical screening and determination of quinazoline alkaloid in *adhatoda vasica*. *Int. J. Pharm. Sci. Rev. Res.* **14(2)**: 115-118.
- Taydae, S.K. and Patil, D.A. (2005). Ethno medicinal Traditions of Tribals of Nandurbar District(Maharashtra). *J. Phytol. Res.* **18(2)**: 251-254.
- Tshibangu J. N., Chifundera R., Kamunsky A. D., Wright, Konig G. M.(2002). Screening of African medicinal plants for antimicrobial and enzyme inhibitory activity. *J. Ethnopharmacol.* **80**: 25-35.

- Vaisakh, M.N. and Pandey,A. (2012). Pharmacognostic study of leaves of *Chromolaena odorata* Linn. *Int. J. Pharm. Res. Dev.* **4(2)**: 33 -37.
- Venkataswamy, R., Mohamad Mubarack, H., Doss, A., Ravi, T.K and Sukumar M, (2010). Ehtnobotanical Study of Medicinal Plants Used by Malasar Tribals in Coimbatore District of Tamil Nadu (South India). *Asian J. Exp. Biol. Sci.* **1(2)**: 387-392.
- Vikrant Arya, Narender Thakur, Kashyap, C.P. (2012). Preliminary phytochemical analysis of the extracts of *psidium* leaves. *J. Pharm. Phytochem.* **1(1)**: 1
- Vlietinck, A.J., Van Hoof, L., Totte, J., Lasure, A., Berghe, D., Rwangbo, P.C., Mvukiyumwani, J. (1995). Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *J. Ethnopharmacol.* **46**: 31-47.
- Wangkhem Sandhya Chanu and Kananbala Sarangthem, (2014). Phytochemical constituents of *justicia adhatoda* linn. Found in Manipur. *Indian. J. Plant. Sci.* **3(2)**: 19-21.
- Watcho, P., Kamtchouing, P., Sokeng, S.D., Moundipa, P.F., Tantchou, J., Essame, J.L., Koueta, N. (2004). Androgenic effect of *Mondia whitei* roots in male rats. *Asian. J. Androl.* **6**: 269–272.
- Wild, S., Roglic, Green A., Sicree R., King H. (2004). Global prevalence of Diabetes: Estimated for the year 2000 and projection for 2030. *Diabetes Care.* **27(5)**: 1047-53.
- Wniger, B. and L. Robinean. (1988). Elements for Carribean Pharmacopoeia. *Proceedings of TRAMIL workshop, Cuba.*
- Yakubu, M.T. (2012). Effect of a 60-day oral gavage of a crude alkaloid extract from *Chromolaena odorata* leaves on hormonal and spermatogenic indices of male rats. *J. Androl.* **33(6)**: 1199-1207.
- Yarapa lakshmi kantha Ramachandra, Chavaan Ashajyothi, Satwadi padmalatha, Supriyakapil shetty thanekar, Shirur Dakappa shruthi. (2013). Antibacterial activity of leaf extracts of *Adhatoda vasica*. *Int. j. Biomed. Pharm. sci.* **7(1)**: 45-47.

Zachariades, C., Day, M., Muniappan, R., Reddy, G.V.P. (2009). *Chromolaena odorata* L. King and Robinson Asteraceae. In *Biological Control of Tropical Weeds using Arthropods*.

Zaika, L.L. (1975). Spices and herbs: their antimicrobial activity and its determination. *J. Food. Safety*. **9**: 97-118.