Department of Biochemistry

T. K. M. College of Arts & Science

(*Affiliated to University of Kerala*)

Kollam-5

April, 2019-22

PHYTOCHEMICAL SCREENING OF

Chromolaena odorata

PROJECT REPORT

Dissertation Submitted to the University of Kerala in partial fulfillment of the requirement for the award of the Degree of Bachelor of Science in Biochemistry

1. **NOORA VASATH** **REG NO:23819142008**
2. **SREEJITH M. S.** **REG NO: 23819142009**
3. **UMA MOL K** **REG NO: 23819142010**
4. **ADARSH S.** **REG NO: 23819142011**
5. **AKHILA SUDEESH** **REG NO: 23819142012**
6. **ANILA SREELEKSHMI K BABU** **REG NO: 23819142014**
7. **ANWAR MUHAMMED** **REG NO: 23819142015**

PHYTOCHEMICAL SCREENING OF

Chromolaena odorata

DECLARATION

We hereby declare that the project titled 'Phytochemical Screening of *'Chromolaena odorata'* is based on the original work carried out by us under the supervision of Dr. Latha.B, Assistant Professor and Head of Department of Biochemistry, T.K.M. College of Arts and Science, Kollam.

We also declare that the project report hasn't been submitted either partly or completely for the award of any other degree or diploma or other similar titles of any other University/ Institutions in India or abroad.

1. Noora Vasath
2. Sreejith.M.S
3. Uma Mol.K
4. Adarsh.S
5. Akhila Sudeesh
6. Anila Sreelekshmi.K Babu
7. Anwar Muhammed

Department of Biochemistry

T. K. M. College of Arts & Science

(*Affiliated to University of Kerala*)

Kollam-5

Date: .....

CERTIFICATE

This is to certify that the dissertation entitled '**Phytochemical Screening of *Chromolaena odorata***' submitted to the University of Kerala in partial fulfilment of the requirements for the award of the degree of Bachelor of Science in Biochemistry, is a record of original research work carried out by the following candidates under my guidance and supervision.

1. Noora Vasath Reg. No. **23819142008**
2. Sreejith.M.S Reg. No. **23819142009**
3. Uma Mol.K Reg. No. **23819142010**
4. Adarsh.S Reg. No. **23819142011**
5. Akhila Sudeesh Reg. No. **23819142012**
6. Anila Sreelekshmi.K Babu Reg No.**23819142014**
7 Anwar Muhammed Reg No.**23919142015**

It is also certified that no part thereof has been presented for the award of any other digreesor diploma or other similar titles of any other university

Dr. Latha.B

External Examiners **Project Guide**

Head of department of Biochemistry

1.

2.

ACKNOWLEDGEMENT

We would like to express our heartfelt gratitude to our guide Dr. Latha. B., HOD of Biochemistry T.K.M. College of Arts and Science, Kollam for her guidance and encouragement that lead us to the successful completion of the work

A special thanks to Dr. Sumayya, Dr. Ansil.P.N, Dr. Hari Sankar, Ms. Soumya S and Ms. Ajinza A., for all their valuable help. We extend our sincere thanks to all the staff members of the college laboratory for their support and assistance.

Our heartfelt thanks to our dear friends for the moral support and help provided for the successful completion of this work. We wish to express our deepest gratitude to our parents for their support and encouragement throughout our work

Above all we are indebted to THE ALMIGHTY for moulding us in the present state and his blessing.

INDEX

LIST OF FIGURES

LIST OF TABLES

1. Introduction

Plants have played a unique holistic role for the provision of food, drugs, clothing, shelter, etc. Natural compounds have been extensively explored for new drug discoveries. Indeed, plants have been used as medicines for more than 5000 years, as a source of antibiotics, antineoplastic, analgesics, cardioprotective, etc., (Chandra et al., 2017; Chen et al., 2015). About 70-90% of the population in developing countries continue to use ancient medicines based on plant extracts. Around 25% of the drugs prescribed world-wide came from plants. Of that 252 considered as basic and essential by the World Health Organization (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors. Natural products have played an important role in the discovery of drugs and therapeutics. The most powerful and promising elements of plants are their secondary metabolites, on which humans depend upon. (Robinson and Zhang, 2011).

The disciplines of ethnobotany and ethnopharmacology define "medicinal plant" as those species used in traditional medicine that contain beneficial elements in healing diseases in humans and/or animals. The objective of ethnopharmacology is to develop a drug to treat patients, and ultimately to validate traditional use of medicinal plants. Throughout human history, the isolation and identification of biologically active compounds and molecules from nature has led to the discovery of new therapeutics, prompting the improvement of the health and pharmaceutical sectors. The exploration of medicinal properties of plants throughout the ages was accomplished principally through careful observation, trial, and error, and accidental discovery, which are beneficial from nutritive and medicinal stand points. Biologically active molecules isolated from the plants revolve around the research and development sector of the pharmaceutical industries as a source of new molecules leading to the development of novel drugs. Significantly, natural products and their derivatives contribute to about 50% of the Food and Drug Administration (FDA) approved drugs. For instance, in the oncology sector, plants have contributed more than 60% of the anti-cancer drugs, directly or indirectly (Pye et al., 2017; Boucher et al., 2017).

The medicinal properties of these plants are mainly attributed to the phytochemicals in them, which are defined as bioactive non-nutrient compounds in fruits, vegetables, grains, and other plants. These are an amazing array of organic chemicals with an enormous diversity of structural types. Many of these phytochemicals are essential for plant growth and development and are widely used by humans due to its health benefits. Phytochemicals are small molecules with diverse chemical profiles and more "drug-like" than synthetic compounds, hence, they are considered as good candidates for the development of drug leads. Phytochemicals, by acting individually or synergistically, helps to reduce the risk for a variety of

chronic and inflammatory conditions. These include atherosclerosis and stroke, myocardial infarction, certain types of cancers, diabetes mellitus, allergy, asthma, arthritis, Crohn's disease, multiple sclerosis, Alzheimer's disease, osteoporosis, psoriasis, septic shock, AIDS, menopausal symptoms, and neurodegeneration (Zhang et al., 2015). India is the largest producer of medicinal plants and is rightly called the "Botanical Garden of the World". Medical information referred to in the old Indian literature includes several medicinal herbs, which have been in use for thousands of years, in one form or the other, under the indigenous system of medicine. In India, 45,000 plant species have been identified, out of which about 15-20 thousand plants are of good medicinal value. However, traditional communities use only about 7000-7500 plants for medicinal purposes. The Siddha system of medicine uses about 600, Ayurveda 700, Unani 700 and modern medicine about 30 medicinal plants for treating a variety of diseases in man and animal. Traditional medicines all over the world are nowadays being re-evaluated by extensive research on different plant species with reference to their therapeutic principles (Madhuri and Pandey, 2008).

Fig.1. ***Chromolaena odorata***

Chromolaena odorata (L.) R.M. King & H. Rob. (Syn: *Eupatorium odoratum* L.) (Asteraceae) is a perennial herb that may reach up to 3 m. The leaves are opposite, deltoid ovate, triangular or lanceolate, achenes with 4 mm long, 4-5 ribbed, bristly on ribs and has many white hairy pappus. This plant is distributed throughout India, tropical Asia and Mexico. This plant is native from Florida through the West Indies and through Central and South America to Argentina (Liogier, 1997). The plant exhibited allelopathic effects and has been reported to cause livestock death (Zachariades et al., 2009).

Traditionally this plant is used in coughs and colds, treatment of skin diseases, wound healing and as a local antiseptic agent (Morton, 1981). Extensive studies of the *C. odorata* have led to identification of

several compounds especially in essential oil from various plant parts. *C. odorata* does not tolerate shade and flourish well in open areas. They can form dense stands and suppress the growth of other plants. This is due to the competition and allelopathic effects. The plant will become invasive in the frost-free areas from medium to arid woodland which are not water-stressed in the growing season. When the plant is dry, it will promote wildland fires (Muniappan, 2000).

In north-eastern India, Siam weed is regarded as a nutrient-demanding early succession species. It takes advantage of the flush of soil that becomes available after a disturbance (such as fire or land clearing for agriculture) and exhibits relatively high foliar natrium, phosphorus and kalium contents (King and Robinson, 1970).

C. odorata blooms annually and is an abundant producer of seeds. Flowering and fruiting begins after the plants are one year old (Lieogier, 1997). The flowers are pollinated by wind, clinging to hair and clothing of animals and humans. The tiny seeds can occur contaminated in imported grass seeds (King and Robinson, 1970).

The individual stems of *C. odorata* will grow for about two years and die near the base of the plant and later will be replaced by new sprouts. The plants' survival skill is very strong-whenever they are being burnt or cut, they can grow back soon. The best current control method is mechanical or hand cutting followed by herbicide treatment. Partial control can be obtained through the use of aggressive cover crops (Muniappan, 2000).

2. Objectives

- Collect the flowers of ***Chromolaena odorata***
- Prepare the water extract of the shade dried flower
- To carry out the phytochemical screening of the water extract of ***Chromolaena odorata***
- To quantify the amount of antioxidant and phenolic compound

3. Review of literature

Asteraceae (or Compositae) is one of the largest family of flowering plants (Pérez-Amador, et al.. 2010) *Eupatorium odoratum* Linn is a shrub of genus of Eupatorium (Compositae). It is also known as *Chromolaena odorata*. It is a shrub belonging to the Asteraceae family. *Eupatorium odoratum* L is a Christmas bush, also known as bitter bush, baby tea, is a scrambling shrub. The plants are maintained by a system of abundant, yellowish, fine lateral roots. The leaves are aromatic when crushed. These flowers are white, flowering from August to October. It is distributed throughout Indian, Indochina and common in open countries. In Myanmar, it can be widely distributed anywhere (Ahmad et al. 1969).

3.1. Scientific Classification

Table.1. Taxonomic classification of *C. odorata*

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Genus	*Chromolaena*
Species	*C. odorata*

Binomial Name : *Chromolaena odorata (L.) R.M.King & H.Rob.*

Synonyms

- *Chrysocoma maculata* Vell.
- *Chrysocoma maculata* Vell. Conc.
- *Chrysocoma volubilis* Vell. Conc.
- *Eupatorium brachiatum* Sw. ex Wikstr.
- *Eupatorium clematitis* DC.
- *Eupatorium conyzoides* Mill.
- *Eupatorium dichotomum* Sch. Bip.
- *Eupatorium divergens* Less.
- *Eupatorium floribundum* Kunth
- *Eupatorium graciliflorum* DC.
- *Eupatorium klattii* Millsp.
- *Eupatorium odoratum* L.
- *Eupatorium sabeanum* Buckley
- *Eupatorium stigmatosum* Meyen & Walp.
- *Osmia atriplicifolia* (Vahl) Sch. Bip.
- *Osmia clematitis* (DC.) Sch. Bip.
- *Osmia divergens* (Less.) Sch. Bip.
- *Osmia floribunda* (Kunth) Sch. Bip.
- *Osmia graciliflora* (DC.) Sch. Bip.
- *Osmia graciliflorum* (DC.) Sch. Bip.
- *Osmia odorata* (L.) Sch. Bip.

Vernacular Names: Siam weed,Christmas bush, jack in the box, devil weed, Communist Pacha (Communist pacha) in Malayalam, common floss flower, rompe saragüey (in Spanish), Abani di egwu or Nsiibilibe (Igbo language), and triffid.

3.2. Geographical Distribution

E. odoratum, also known as *C. odorata* is a native of tropical America, from south Florida, USA to northern Argentina, and it is found in t habitats below 1000m altitude and receiving 1200mm rainfall (McFadyen and Skarratt 1996). The distri- bution of E, odoration is limited by its intolerance to frost and to a lesser extent to low rainfall (Gareeb et al., 2004). It has invaded the old world tropics from West to South Africa to South and South-East Asia and the Pacific Region (McFadyen, 1989; McFadyen and

Skarratt, 1996; Baskin, 2002). It has spread to Australia, Cambodia, Indonesia, Malaysia, Philippines, Thailand and Vietnam in the Pacific region and Mauritius in the Indian Ocean. It is a serious weed in 23 countries of the World including India, Malaysia, Sri Lanka and Thailand (Holm et al., 1977). In the Philippines, some villages were deserted due to non productivity of the land infested with this weed (Pancho and Plucknett, 1971).

E. odoratum probably got introduced to India and Myanmar from Jamaica by seeds in the ballasts of cargo boats anchoring at Singapore after Roxburgh's time. From there, the plant found its way to lower Myanmar, gradually encroaching further inlands in eastern Himalaya, eastern and southern India, extending up to Bihar in upper Gangetic plains, in Sikkim and in western Himalaya (Dehradun) (Raizada, 1976). *E. odoratum* is a dominant weed at lower elevations in north-eastern region of India and Western Ghats (Rao, 1977). It also infests tea,teak and rubber plantations and vegetable crops in the country.

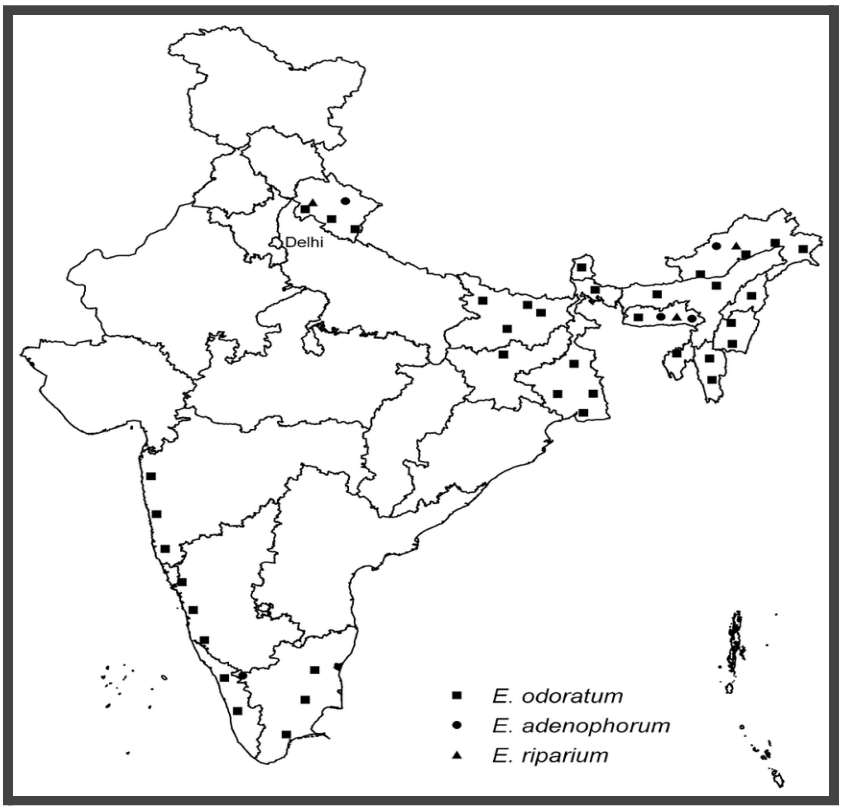

Fig.2. Distribution of *Chromolaena* in India

3.3. Botanical Description

Siam Weed is a big bushy herb or subshrub with long rambling (but not twining branches. In open areas it spreads into tangled, dense thickets up to 2 m tall, and higher when climbing up vegetation. Many paired branches grow off the main stem. The base of the plant becomes hard and woody while the branch tips are

soft and green. The leaves are arrowhead-shaped, 5-12 cm long and 3-7 cm wide, with three characteristic veins in a 'pitchfork' pattern. They grow in opposite pairs along the stems and branches. As the species name 'odorata' suggests, the leaves emit a pungent odour when crushed.

Fig.3. Diagrammatic sketch of *C. odorata*

Clusters of 10-35 pale pink mauve or white tubular flowers, 10 mm long, are found at the ends of branches. The seeds are dark coloured, 4-5 mm long, narrow and oblong, with a parachute of white hairs which turn brown as the seed dries. Siam weed is native to Tropical America, but is now naturalized throughout the tropics.

E. odoratum,, called Siam weed, Christmas weed or bitter bush (King and Robinson, 1970; Howard, 1989), is a perennial shrub or large herb forming dense tangled bushes of up to 2.0 m height but reaching up to 6 m when climbing up the trees (Roder et al., 1995; Riddoch et al., 1991). Stem is terete, striate. with soft hair. Petiole is 15 cm long and densely hairy. Leaves measure 6x3 cm across and are ovate to ovate rhomboid, clothed with slender soft hair on dorsal and dense hair on the ventral surface, cuneate at base, margins coarsely dentate or dentate serrate and apex acuminate. Heads are corymbose, homogamous with 30 tubular flowers, in volucral, bracts numerous, many serrate and outer shorter. Cypsela is five-angled (Raizada 1976). The leaves are opposite, three nerved, are deltoid to ovate-lanceolate and are aromatic when crushed. The inflorescences are corymbs of cylindrical heads located on the terminal lateral branches. There are about 15 to 25 tubular florets per head, which are white, lavender, pink or blue in

colour. The seeds have brownish gray to black achene that is 4mm long and a pale brown papus 5 or 6mm long (Liogier, 1997).

3.4. Chemical Composition

Several chemical analyses of *C. odorata* L. have been undertaken that have identified constituents including monoterpenes, sesquiterpenes hydrocarbons, triterpenes/steroids, alkaloids and flavonoids (Heiss et al., 2014). The leaves of this plant have been found to be a rich source of flavonoids including quercetin, sinensetin, sakuranetin, padmatin, kaempferol and salvagenin (Torrenegra and Rodriguez, 2011). The leaves of *C. odorata* also contain the highest concentration of allelochemicals isolated from a plant (Akinmoladun et al., 2007). A study in Vietnam revealed that the aqueous extract of the leaf contained flavonoids (salvigenin, sakuranetin, isosakuranetin, kaempferide, betulenol, 2-5-7-3 tetra-o methyl quercetagetin, tamarixetin, two chalcones and odoratin and its alcoholic. compound), essential oils (geyren, bornyl acetate and B-eubeden), saponin triterpenoids, tannins, organic acids and numerous trace substances (Zhang et al., 2012). Another study by Heiss et al (Heiss et al., 2014) demonstrated that the crude ethanol extract of *C. odorata* contains phenolic acids (protocatechuic, p-hydroxybenzoic, p-coumaric, ferulic and vanillic acids] and complex mixtures of lipophilic flavonoid aglycones (flavanones, flavonols, flavonoids and chalcones). To date, studies on *C. odorata* have resulted in the isolation of 17 compounds, including 5aa,6.9.9a8,10-pentahydroxy-10B-hydroxy-7 methyl anthra[1,2-d] [1,3]dioxol-5-one,
1,2-methylenedioxy-6-methylanthraquinone,3-hydroxy-1,2,4-trimethoxy-6-methylanthraquinone,
3-hydroxy-1,2-dimethoxy-6 methylanthraquinone and 7-methoxy-7-epi-medioresinol, as well as 12 known compounds including odoratin, 38-acetyl oleanolic acid, ursolic acid, ombuin, 4,2' dihydroxy-4',5',6'-trimethoxy chalcone, (-)-pinoresinol, austrocortinin, tianshic acid, cleomiscosin D, (-)-medioresinol, I-1-syringaresinol, and cleomiscosin A (Zhang et al., 2012).

Table.2.1. Chemical Constituents in aqueous Flower Extracts of *C. odorata*

Part of plant	Chemical constituent	References
	● 3,5,4-trihydroxy-7-methoxyflavanone	Odunbaku and Ilusanya, 2008

Flower (aqueous extract)	● 5,7,3''''''-trihydroxy-5-methoxyflavanone	
	● 3,5,7-trihydroxy-4-methoxyflavanone	

Table.2.2. Chemical Constituents in Flower Extract of *C. odorata*

Part of plant	**Chemical constituent**	**References**
Flower	● Akuranetin	Suksamrarn et al., 2004; Pisutthanan et al., 2006
	● Persicogenin	
	● 5,6,7,4'-tetramethoxyflavone	
	● 4'-hydroxy-5,6,7-trimethoxyflavone	
	● 2-hydroxy-4,4,5,6-trimethoxy chalcone	
	● 4,2-dihydroxy-4,5,6-trimethoxyflavone	
	● Acacetin	
	● Luteolin	
	● 5, 7-dihydroxy-6-4-dimethoxyflavone	

Table.2.3. Chemical Constituents in dichloromethane extract of Whole plant

Part of plant	Chemical constituent	References
Whole plant- above ground (dichloromethane extract)	● 2'-hydroxy-3,4,4,5,6-pentamethoxy-chalcone	Barua et al., 1978
	● 2,4-dihydroxy-4,5,6-trimethoxy chalcone	
	● Scutellarein tetramethyl ether	
	● Sinensetin	
	● 2-hydroxy-4,4,5,6'-trimethoxy chalcone	

Table.2.4. Chemical Constituents in ethanol and methanol extract of Whole plant

Part of plant	Chemical constituents	References
Whole plant (ethanol and methanol extract)	● Aromadendrin 4" methyl ether	Ling et al., 2007a; Suksamrarn et al 2004; Ling et al., 2007b
	● Eriodictyol 7,4-dimethyl ether	
	● Naringenin 4'-methyl ether	
	● Taxifolin 4'-methyl ether; taxifolin 7-methyl ether	

	● Quercetin 7,4-dimethyl ether	
	● Kaempferol 4'-dimethyl ether	
	● Quercetin 3-0-rutinoside	
	● Quercetin 4'-methyl ether	
	● Quercetin 7-methyl ether	

Table.2.5. Chemical Constituents in ethanol extract of Leaves

Part of plant	**Chemical constituent**	References
	● Tamarixetin	
	● Trihydroxy Monomethyl Flavanone	
	● Pentamethoxy Flavone	
	● Dihydroxy Trimethoxy Chalcone	
	● Eupatillin; 5,6,7,4'-Tetramethoxyflavone	
	● 5-Hydroxy,7,3,4'-tetramethoxyflavone	

Leaves (ethanol extract)	● Kaempferide	Phan et al., 2001b
	● Protocatechuic acid	
	● p-Coumaric acid	
	● p-Hydroxybenzoic acid	
	● Ferulic acid	
	● Vanillic acid	
	● Sinensetin	
	● Rhamnetin	
	● Tetrahydroxy Monomethyl Flavanone	

Table.2.6. Chemical Constituents in methanol extract of Root and Flower

Part of plant	**Chemical constituent**	**References**
	● 7-angelou retronecine	
	● 9-angelou retronecine	

Root and flower head (methanol extract)	• Supine	Biller et al., 1994
	• Intermidine	
	• Lycopsamine	
	• Rinderine	
	• Echinatine	
	• 3'-Acetylpyridine	

Table.2.7. Chemical Constituents in aqueous extract of Whole plant

Part of plant	**Chemical constituent**	**References**
Whole plant (aqueous extract)	• Alpha-pinene	Dieneba et al., 1992
	• Limonene	
	• p-Cymene	
	• cadinene	
	• Beta-caryophyllene	

	• Camphor	
	• Cardinal	
	• Germacrene D	

Table.2.8. Chemical Constituents in petroleum extract of Whole plant

Part of plant	**Chemical constituent**	**References**
Whole plant (cold light petrol b.p. 60-80°)	Lupeol	Talapatra et al., 1974
	beta-amyrin	
	Salvigenin	

3.5. Traditional Medicinal Use

Leaf extracts of *C. odorata* added with salt are used as gargle for sore throat and colds. The leaves are also used to scent aromatic baths. By adding copious amounts of organic matter to soil, it is reported that it may reduce the population of nematodes (M'Boob, 1991). The leaves are also used as emergency medication. By pounding the leaves till fine and app1lied to the wound, it can stop the bleeding of the wound. Furthermore, it can be used during emergencies where we can crush the leaves by hand, mix with some saliva and apply it on the wound (Muhamad and Mustafa, 2004).

In traditional medicine, a decoction of the leaves is used as a cough remedy and as an ingredient with lemongrass and guava leaves for the treatment of malaria. In Thailand, the juice of the leaf is used as a haemostatic on wounds and anti-inflammatory. While, decoction of the flowers is used as tonic, antipyretic and heart. tonic (Bunyapraphatsara and Chokechajiaroenprom, 2000). In Vietnam, fresh leaves

or decoction of the leaves have been used for the treatment of leech bite, soft tissue. wounds, burnt wounds, skin infection and dental-alveolitis (Phan et al., 2001b; Ling et al., 2007).

In some studies, it helps to reduce the desire to smoke, cures fever, coughing. jaundice and stomach ache. The fresh leaves and extract of *C. odorata* are used as traditional herbal treatment in developing countries for burns, soft tissue wounds and skin infections (Phan et al., 2001a). Other medicinal uses include antidiarrhoeal, astringent, antispasmodic, antihypertensive, anti inflammatory and diuretic (Iwu, 1993). Boiling of the herb is employed as a cough reliever in ancient drugs, and as an ingredient with lemongrass and guava leaves for protozoal infection treatment.

The decoction of the fresh leaves has been used traditionally in many tropical countries as a treatment for leech bite, tissue wounds, burns and skin infections (Le, 1995). The leaf extract of *C.odorata* is also known to inhibit the growth of some bacterial strains (Bamba et al., 1993). The extracts have been reported to show potent activity against *Mycobacterium tuberculosis* H 37 Ra (Collins & Franzblau, 1997). The aqueous extract and the decoction from the leaves of this plant have been used since then throughout Vietnam for the treatment of soft tissue wounds, burn wounds, and skin infections (Phan et al., 1999)

3.6. Pharmacological Properties of *Chromolaena odorata*

Herbal plants are known to be rich sources of phytochemical ingredients that contribute to healthcare management (Shakya & Kumar, 2016). *C. odorata* has been commonly and widely used in traditional medicine because of its properties that can give therapeutic effects on the body. The leaf extracts of *C. odorata* have been shown to possess antioxidant, anti inflammatory, analgesic, antimicrobial, cytoprotective and many other medicinally significant properties (Vaisakh & Pandey, 2012).

The phytochemical components of *C. odorata* include alkaloids, flavonoids, flavanone, essential oils, phenolics, saponins, tannins, and terpenoids. The other essential constituents of this plant are chromomoric acid, quercetagetin, and quercetin, all of which contribute to its medicinal properties (Sirinthipaporn & Jiraungkoorskul, 2017). For instance, it has been reported in several studies that these phytochemicals are able to exhibit a wide spectrum of pharmacological activities including antioxidant activity, hypoglycemic and hypocholesterolemic effects in animals (Rahman et al., 2008) as well as modulation in wound healing stages. (Vijayaraghavan et al., 2018).

3.6.1. Antimicrobial Activities

3.6.1.1. Antibacterial Properties

The *C. odorata* leaves extracts from ethanol, methanol, and hexane extraction have been reported to exhibit strong inhibitory effects against both Gram-positive (*Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Propionibacterium acnes*) and gram-negative (*Proteus vulgaris*) bacterial strains (Hanphanphoom & Krajangsang, 2016).

In addition to leaves extract, hexane extract of *C. odorata* stem showed greater inhibitory activity against *Pseudomonas aeruginosa*, *B. cereus* and *Klebsiella pneumoniae*, while hexane root extract showed high inhibitory activity against *Enterococcus faecalis* and *K. pneumoniae*. The findings also reported that the ethanolic and methanolic leaf extracts produced high extraction yield and high contents of both total phenolics and flavonoids. Besides, it has been reported these extracts exhibited good antibacterial activity against the gram positive bacteria *Staphylococcus S. aureus*, *S. pyogenes* and *Staphylococcus epidermidis* and the gram-negative bacteria *P. vulgaris*. The results obtained suggested that the ethanolic, methanolic and hexane leaves extracts of *C. odorata* are promising to be further developed in treating bacterial skin infections (Hanphanphoom & Krajangsang, 2016).

Furthermore, it was reported that *C. odorata* leaves extracts from four different. solvents: cyclohexane, dichloromethane, ethyl acetate and butanol displayed antibacterial activities against four bacteria that cause intestinal tract infection including *Klebsiella oxytoca*, *Salmonella enterica*. *Shigella sonnei* and *Vibrio cholera*. This further validates the traditional use of this plant in the treatment of intestinal infectious diseases (Omokhua et al., 2016).

A recent study by Udayaprakash et al., demonstrated that acetone extract of *C. odorata* exhibited high inhibitory activity against *S. aureus* and *P. aeruginosa*. Meanwhile, ethyl acetate extract of *C. odorata* recorded the maximum zone of inhibition against *Bacillus subtilis* and chloroform extract demonstrated strong. inhibition against *Streptococcus mutans*. It is worth noting that the extracts of the directi solvent extraction method were shown to possess better antibacterial compounds when compared to the sequential extraction method (Udayaprakash et al., 2019).

3.6.1.2. Anthelmintic Properties

Helminth infection is currently the most widespread infection found in human beings. They pose a significant threat to the population of the world which leads to starvation, anemia, eosinophilia, and

pneumonia. The gastrointestinal helminths are immune to anthelmintic medicines currently available, so there is a major threat in the treatment of helminth diseases, hence a growing demand for natural anthelmintic. Consequently, the present research carried out by Debashisha Panda et.al, (April 2010) on various solvent extracts of *Chromolaena odorata* leaves was carried out for the anthelmintic operation. The anthelmintic action was experimented with within an adult Indian earthworm, "*Pheretima posthuma*" as it has physiological and anatomical similarity within intestinal roundworm parasites of human beings. The investigation about this activity revealed that methanol extract endowed anthelmintic action in comparison to other extracts such as the petroleum ether and ethyl acetate. The extract capacity was determined to be inversely proportional to the time taken for worm paralysis/death (Panda et al., 2010).

Some synthetic phenolic anthelmintics, e.g. niclosamide, oxyclozanide, bithionol, nitroxynil, etc, are shown to interfere with energy generation. in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997). Tannins are polyphenolic compounds (Smith, 1962). It is possible that tannins contained in the extract of Eupatorium odoratum produced similar effects. In another study, polyphenols from bryophytes were shown to have anthelmintic activity against *Nippostrongylus brasiliensis* (Gamenara et al., 2001). Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal (Athnasiadou et al., 2001) or glycoprotein on the cuticle of the parasite (Thompson & Geary, 1995) and cause death. Several authors have reported that an increase in the supply of digestible protein does improve the resilience and. resistance of sheep to gastrointestinal nematodes (Coop & Holmes, 1996). Tannin containing plants increase the supply and absorption of digestible protein by animals (Waller et al., 2001). This is achieved by formation of protein complexes in the rumen by tannins, which later dissociate at low pH in the abomasum to release more protein for metabolism in the small intestines of ruminant animals (Wang et al., 1994). In addition, tannins or their metabolites have a direct effect. on the viability of the pre parasitic stages of helminths. Other phytochemicals reported to have an anthelmintic effect include essential oils (Persia et al., 2002), flavonoids and terpenoids (Lahlou, 2002).

3.6.1.3. Antimalarial Action

Malaria is a deadly disease caused by Plasmodium parasites and transmitted through the bites of infected female Anopheles mosquitoes. Despite collaborative efforts, 214 million cases of malaria and 423,000 deaths were reported in 2015 (WHO, 2016). The management and control of malaria are challenged due to resistance of malaria parasites to most antimalarial drugs. Artemisinin-based combination therapy (ACT) is currently the most widely used treatment regimen for malaria (WHO, 2008). The combination therapy is

widely advocated because it produces rapid clinical and parasitological response, and may circumvent or delay resistance (Bukirwa and Orton, 2005). Similarly, folk medicines often use a combination of plants in treating a series of infections. Medicinal plants contain numerous compounds which serve as potential drug sources for human disease management (Barliana et al., 2014). *Chromolaena odorata* (L.), a perennial straggling shrub with simple leaves oppositely arranged, is from Asterad antimalarial medicinal plant family and commonly called Siam weed. It is used as an antimalarial medicinal plant (Nisit et al., 2005; Ukpai and Amaechi, 2012).

Table.3. Biological activity of different plant parts of *C. odorata*

Part of plant	**Biological activity**	**Reference**
Aerial part (aqueous extract and essential oil)	● Anti-malarial	Pisutthanan et al., 2006
	● Anti-inflammatory	
	● Antibacterial	
Whole plant (ethanol, dichloromethane and methanol extract)	● Analgesic	Irobi, 1997; Odunbaku and Ilusanya, 2008; Oweyele et al., 2008
	● Anti-inflammatory	
	● Antipyretic	
	● Antibacterial	

Leaf (Methanol, ethanol and aqueous extract)	● Antioxidant	Ling, 2006; Phan et al., 2001a; Phan et al., 2001b; Akinmoladun et al., 2007; Dieneba et al., 1992
	● Anti-inflammatory	
	● Wound healing	
	● Anti-staphylococcal	
Flower	● Antibacterial against *Mycobacterium tuberculosis*	Suksamrarn et al., 2004
	● Anticancer	

3.7. Therapeutic Attributes

3.7.1. Antioxidant activity

Research work on the ethanolic and methanolic extract of plant *C. odorata* leaves by Bhargava et.al, was tested for antioxidant function and free radical scavenging. The extract's ability to scavenge nitric oxide, hydroxyl radical, and 1,1-diphenyl-2picrylhydrazyl (DPPH) was used in this study to determine its antioxidant property. For ethanolic extract, methanolic extract and vitamin C, the DPPH radical inhibition (percent) was there. Furthermore, ethanol and methanol extract also demonstrate extensive radical scavenging of nitric oxide and hydroxyl radicals. The leaves methanolic and ethanol extract reveals the drug's anti-oxidant effects. The extract's free radical scavenging behavior was tested based on its capacity to scavenge the DPPH. This method is particularly important to provide details on the reactivity of organic compounds with stable free radicals due to the unusual number of electrons. The research result shows

that the leaves of *Chromolaena odorata* have ethanol content (Angitha, A et al., 2021). The DPPH system scavenged free radicals by *odorata* (L). It shows a robust color spectrum, optical phenomena (deep violet color) at 517 nm. The DPPH absorption bleaching indicates the power of the test drugs to free radical scavenging (Bhargava et al., 2013).

Antioxidant is an important substance in the body that works by protecting the body from any harm or injury that is eventually caused by oxidative stress due to free radicals (Rao et al., 2010). The natural antioxidants present in the *C. odorata* plants such as polyphenols play an important role in preventing the body from oxidative damage (Rao et al., 2010). This is because the ideal chemical structure of polyphenols compounds makes the plants more effective as an antioxidant against a free radical scavenging activity as compared to any other compounds such as ascorbate and tocopherols (Rao et al., 2010). *C. odorata* was also able to stimulate the production of antioxidants at the wound site by avoiding tissues from oxidative damage and providing a favourable environment for tissue healing (Vijayaraghavan et al., 2017). Basically, the reactive intermediates (ROS) can cause a delay in wound healing. Phytochemical compounds of *C. odorata* such as alkaloids and flavonoids were also shown to have antioxidant activity that can reduce or regulate the oxidative damage caused by ROS generation. Furthermore, *C. odorata* could enhance antioxidant enzyme levels, in which these enzymes can quench the superoxide radical and avoid the free radical mediated damage to cells. Thus, it can support the wound healing mechanism (Vijayaraghavan et al., 2017).

In addition, the polyphenols are able to exhibit antioxidant properties due to the high reactivity of polyphenol compounds that can act as either an electron or hydrogen donors. and its ability to stabilise and remove the The unpaired electron (Rao et al., 2010). The phenolic compound that can be found in crude extracts of *C. odorata* essentially can work as a metal chelator, reducing agent, free radical quenchers, as well as a hydrogen donor. This function is due to their redox properties that enable the phenolic compounds in this plant to exhibit antioxidant properties (Rao et al., 2010).

The common solvents for extraction of *C. odorata* plants that exhibit antioxidant activity include chloroform, ethanol. methanol, and petroleum ether. Overall, the ethanol extract of the leaves demonstrated the most effective antioxidant property (Omokhua et al., 2016).

3.7.2. Anti-inflammatory Activity

The *C. odorata* plants also have been shown to exhibit anti-inflammatory properties. It was reported that the aqueous and ethanolic extracts of *C. odorata* could retard the inflammatory reaction. The

inflammatory activities exhibited by C. odorata may be due to the presence of anti phenolic compounds in these extracts (Omokhua et al., 2016). Besides, it has also been shown that the chloroform extract of *C. odorata* exhibits significant inhibition on the production of nitric oxide (Rao et al., 2010). Nitric oxide (NO) is a free radical produced naturally in the body, however overproduction of NO may cause many types of the inflammatory process in the body (Rao et al., 2010). Therefore, inhibition of NO could prevent or reduce the inflammatory effects in the body (Rao et al., 2010).

Furthermore, the acid derivatives such as coriolic acid, linoleamide and didehydrocoriolic acid from the extracts of *C. odorata* were shown to be a natural inhibitor of NF-kB (nuclear factor kappa-light-chain enhancer of activated B cells). NF-KB is a crucial mediator of inflammation. Therefore, inhibition of NF-KB transcriptional factor is considered a therapeutic target for anti-inflammatory treatment by the fatty acid components of *C. odorata* (Hanh et al., 2011).

Flavonoid compounds such as chalcones were reported to be associated with a potent anti-inflammatory activity, which is also targeting the NF-kB signaling pathway. Chalcones were shown to exhibit anti-inflammatory activity by suppressing the activation of NF-KB signaling pathways, resulting in the reduction of proinflammatory cytokines in LPS-activated macrophages. This action justifies the role of chalcone in reducing inflammation (Dhar et al., 2018).

In addition, based on the anti-inflammatory test conducted, the aqueous extract of *C. odorata* was able to consistently produce high levels of anti-inflammatory activities in acute and chronic models of inflammation (Owoyele et al., 2006). It is postulated that the flavonoid compounds of *C. odorata* may be responsible for the anti-inflammatory activities in the body (Owoyele et al., 2006).

3.7.3. Analgesic Activity

Substances that relieve pain can be defined as analgesics (painkillers). Analgesics works through various mechanisms and functions either centrally (opioids receptor agonism) or peripherally. In recent years, there has been an impetus on the use of traditional medicinal plants with analgesic effects worldwide due to its natural origin and lesser side effects (Rauf et al., 2017). A study by Owoyele et al. reported that *C. odorata* ethanolic extraction was shown to exhibit analgesic activity through hot plate latency assay and formalin paw licking tests.

The effectiveness of *C. odorata* extracts. to exhibit the analgesic properties might be due to its active phytochemical constituents (Onoja et al., 2016). The main active constituents responsible for the analgesic property include glycosides, terpenes, flavonoids, steroids. tannins, alkaloids as well as saponins (Nudo &

Catap, 2012). The effectiveness of *C. odorata* plant extract on the analgesic properties was reported to be approximately similar to the pentazocine drug. Pentazocine is an opioid pain medication which is sometimes referred to as a narcotic This drug also works as an analgesic which eventually interacts with k receptors which in turn causes sedation (Onoja et al., 2016).

3.7.4. Treatment of Skin Infection

C. odorata is a traditional medicinal plant that has been used for its many medicinal properties including external application to treat skin infections. For instance, the West and Central African exploited the plant for the management of a wide range of medical conditions, despite this being a non-native species. Besides, it was reported that the stem extract of *C. odorata* plants has been demonstrated to be effective for the treatment of skin infections, particularly caused by the *Propionibacterium acnes* (Pandurangan et al., 2015).

The extract from the leaves of *C. odorata* has also been widely used in countries such as Vietnam and other tropical countries to treat skin infections and rashes (Wang et al., 2014). Besides, in other countries such as Thailand and India, this plant has been extensively used as a traditional herb to treat skin infection (Vijayaraghavan et al., 2017).

3.7.5. Treatment of Stomach Related Problems

The species of *C. odorata* plants, found in the West of Africa and Asia were also found to be useful in reducing stomach-ache (Omokhua et al., 2016). The phenolic compound in *C. odorata* leaves extract has been shown to prevent internal bleeding from diathesis and stomach ulcers. Equally important, is that this compound also preserves the keratinocytes from being damaged and reduces the internal bleeding from the stomach ulcer (Paul et al., 2018).

In addition to phenolic compounds, the presence of other active constituents such as flavonoids and tannins have demonstrated to be essential in arresting internal bleeding from stomach ulcers and minimising the bleeding diathesis in heparin-induced mouse models. Moreover, oral administration of *C. odorata* extract was proven to protect the bone marrow cells from busulfan, thereby elevating the platelet count and improving thrombocytopenia conditions (Paul et al., 2018).

3.7.6. Wound Healing

Substances that relieve pain can be defined as analgesics (painkillers). Analgesics works through various mechanisms and functions either centrally (opioids receptor agonism) or peripherally. In recent years, there has been an impetus on the use of traditional medicinal plants with analgesic effects worldwide due to its natural origin and lesser side effects (Rauf et al., 2017).

The presence of the phenolic compound in the *C. odorata* leaf extracts works as an antioxidant, which helps increase the efficacy of *C. odorata* in wound healing. This antioxidant property works by increasing the efficiency of preserving the growth of keratinocytes and fibroblasts on the wounds (Sirinthipaporn & Jiraungkoorskul, 2017). The presence of several active phytochemical compounds possesses a synergistic wound healing activity. It has been proven that the active constituents from the *C. odorata* extract are able to enhance and improve wound healing in laboratory animals including rats (Vijayaraghavan et al., 2017). Besides, there were no adverse side effects such as exudate, wound haemorrhage, inflammation, or oedema when *C. odorata* leaves extracts were used for the treatment of wound healing. Based on a study by Vijayaraghavan & Rajkumar (2017), the most potent concentration of *C. odorata* for wound treatment is at 5% w/w, where it has been proven to heal the area of the wounds significantly faster than control (petroleum jelly) and Betadine-treated groups. In addition, the aqueous extraction of *C. odorata* has been found to accelerate healing in excision wound model in rats (Vijayaraghavan et al., 2017).

The *C. odorata* plant extracts can induce wound healing primarily by enhancing the regulation of thromboxane synthase, heme oxygenase 1, and anti-platelet aggregator genes. On the other hand, the anti-platelet aggregator which is matrix metallopeptidase 9 (MM9) was reduced with the treatment using *C. odorata* leaf extracts (Sirinthipaporn & Jiraungkoorskul, 2017).

3.7.7. Antidiarrheal Activity

The researcher Mayela Nkouka S.H.J assesses the antidiarrheal activity of the aqueous extract of *C. odorata* leaves. The antidiarrheal effect was assessed for diarrhea induced by the castor oil, the charcoal test (intestinal transit time), and the accumulation of the castor oil induced intestinal fluid. The results obtained show that the aqueous extract at the doses used significantly reduces the emission frequency, the amount, and the appearance of the faces induced by the castor oil. The aqueous extract of *C. odorata* does not significantly decrease the intestinal movement but on the other hand, greatly reduces the concentration of fluid in the castor oil mediated intestine. *C. odorata* aqueous extract has an antidiarrheal effect which could be explained by interfering with the electrolyte secretion mechanisms. These results would support

the use of the plant in conventional diarrhea therapy. Albino rats and albino mice of both sexes are used for evaluation of this behavior (Ogbonnia et al., 2010).

3.7.8. Cytoprotective Activity

The experiment conducted by Nurjannah et al., 2006, proved their effectiveness as an antiulcer when used orally. For this study, *C. odorata* extract was provided for use in the treatment of stomach ulcer lacerations in conjunction with honey. Extract of the *C. odorata* was also done. Such tests have shown the presence in the extract of a variety of compounds such as acacetin and luteolin. Such mixtures have demonstrated themselves for their important action against small human breast cancer and lung cancer (Kanase & Shaikh, 2018).

3.7.9. Anti-diabetic Activity

The study, conducted by Oluyemisi Omotayo Omonije et.al, therefore examines the anti diabetic activities of the *Chromolaena odorata* methanolic extract. The extract also enhances glucose transport in a dose-dependent manner. There was a substantial dose dependent rise of whole root extract of *Chromolaena odorata* methanol in *in-vitro* anti-diabetic practice. This inhibitory activity of the extract might be attributed to the presence of phytochemical antioxidants including: flavonoids, tannins, and saponins that are reported to inhibit a-amylase activity and thus protect the B-cell integrity from the event of type 2 diabetics with insulin resistance (Omonije et al., 2019). In accordance with the research which was conducted by Vijayaraghavan et al. (2013), said that after doing phytochemicals analysis of this siam weed leaves was known that this plant contains various antioxidant compound such as tannin, saponin, flavonoid, betacyanins, quinones, glycosides, cardiac glycosides, terpenoid, phenol, cumarin, steroid, and alkaloid. In this research also was done the phytochemicals test qualitatively and 4 compounds such as flavonoid, alkaloid, saponin, and tannin. According to Marianne et al. (2014), flavonoid is known as inhibition of hydroxy and superhydroxy radicals which can protect the membrane lipid of B cells' pancreas toward damaging reactions. According to Widowati (2008), there are some plant mechanisms in lowering blood glucose' level that is the plant has ability as astringent which can precipitate the protein of intestinal mucous membrane and form a layer which protects the intestine, thus can inhibit the glucose' supply and increasing rate of glucose in blood is not too high. Moreover, there is also a plant which decreased blood glucose level by accelerating the secretion of glucose from circulation and by accelerating blood circulation that is through the work of heart and excretion of kidney, thus the secretion rate of urine increased. And there are also plants which accelerate the secretion of glucose through increasing metabolism or entering into fat deposit. This process involves pancreas to produce insulin.

According to Subroto (2006), there are 5 activities of plants in healing diabetes. There is a plant with activity to inhibit alpha-glucosidase (inhibitor), the plant with activity to stimulate insulin secretion, there is also a plant with activity to repair the function of insulin, there is also plant which has double activities such as to inhibit alpha-glucosidase and to repair the function of insulin, and also a plant with complex activities such as to decelerate food digestion, to decrease the absorption rate of carbohydrate into the liver circulation, to influence the transport of glucose which is mediated by Nitric Oxide (NO) and to modulate the insulin secretion which mediated by NO. Decreasing of blood glucose occurs because there is antioxidant compound activity which is contained in siam weed leaves such as flavonoid, saponin, tannin, and alkaloid.

3.8. Economic Uses

C. odorata is used as a green manure in Cambodia in rice fields and for black pepper cultivation (Garry 1963, Litzenberger and Lip 1968). However, the manure of this weed was found to be poisonous and so may increase the crop production indirectly by preventing the attack of nematodes and pathogens on the crops. Mercury-binding peptides from roots, stems and leaves of Hg-treated *E. odoratum* plants were isolated. The high concentration of mercury in the leaves indicate that it has a high potential as a phyto-remediation agent of inorganic mercury (Velaco- Alinsug et al. 2005).

C. odorata has been used in traditional medicine in Asia for a long time; it is mentioned that it may probably help in blood clotting. However, Soogarum et al. (2005) reported that it has no effect on prothrombin time and activated partial thromboplastin time. Apori et al. (2000) showed that *C. odorata* has good potential for feeding livestock due to its high crude protein, low fibre and low extractable phenolic contents. Its leaves can be used as an ingredient for formulating animal feeds. However, all parts of odorata contain high levels of pyrrolizidine alkaloids. (Biller et al. 1994) which renders it unpalatable for grazing (McFadyen, 2003). If cattle goats graze on it, the alkaloids progressively destroy their liver and the animals die (Pancho and Plucknett 1971). *C. odorata* is known to harbour a number of insects and mites injurious to other crops in Asia as reported by Bennett and Rao (1968), Joy et al. (1979), Ramani and Haq (1983) and Muniappan and Viraktamath (1986).

The flowers of *Eupatorium odoratum* show larvicidal, insecticidal and a slight range of anti-bacterial activities. We also detected the presence of an enzyme polyphenol oxidase (PPO) and partially purified the enzyme from flowers. The polyphenol oxidase has a number of applications in industries. PPO can be used in the dye industry as a colouring agent, it is also used in the food industry. It is the reason behind the colouring of black tea and cocoa. PPO is also can be used to cleave the phenol ring thus it can be used to

degrade petroleum compounds that cause soil pollution and so it is applicable in preventing bioremediation also.(Deepa and Jofeena, 2015)

3.9. Uses in Agricultural Field

The threat of *C. odorata* to agriculture has been a global concern and studies have been done around the world to effectively control the menacing weed. However, an evaluation of the progress made has revealed that, through many years of research efforts that had been done, the problem of *C. odorata* has remained unsolved (Ambika and Jayachandra, 1980; Ooi et al., 1998).

Despite claims of *C. odorata* being a menacing weed, there are also some research that shows positive contribution of *C. odorata* to the agricultural sector. Research by Bomikole revealed that using *C. odorata* leaf meal in rabbits' diet has a nutrient profile that is similar to a concentrated feed. Therefore, the leaves of *C. odorata* can be used as an ingredient for formulating animal feed. The *C. odorata* leaf meal is reported to make the production of rabbits more economical and encourage production among farmers due to availability of this feed resource (Bamikole et al., 2004).

Another research by Apori et al., also confirmed that the leaves of *C. odorata* have high nutritive values and have the potential to be used as protein supplements to ruminants. Their chemical analysis reported that the leaves of *C. odorata* are high in protein content with little or no presence of phenolic antinutritive factors (Apori et al., 2001).

A different study by Offor and Okonye shows that *C. odorata* is useful in soil fertility restoration by providing essential constituents needed for plant growth and protection. It is reported that the leaves of *C. odorata* have potentials for protecting and maintaining optimum growth for plants in polluted environments (Offor and Akonye,2006).

In Indonesia, a research by Kumalasari et al., reported that the mulch of *C. odorata* improves the growth of corn (Zea mays L.). Their research involved the application of *C. odorata* mulch on the planting ground of corn. They concluded that the mulch of *C. odorata* improved the content of the mineral phosphorus and nitrogen in the soil.

4. Qualitative Analysis of the Aqueous Extract of *Chromolaena odorata*

4.1. Materials

4.1.1. Plant material

Samples used in the study were flowers of *Chromolaena odorata* collected from Kollam district, Kerala, India. The plants were collected during the months of January to March. Plant material was washed several times with tap water and once with distilled water and allowed to shade dry at room temperature for 3-7 days. Dried plant materials were powdered with the help of a blender and stored in an airtight container. This powder is used for extraction analysis.

4.1.2. Chemicals

- Mayer's reagent.
- Wagner's reagent
- Dragendorff's reagent.
- Basic Lead acetate.
- Ferric chloride solution.
- Potassium dichromate.
- Sodium nitroprusside in pyridine.
- Lead acetate
- Chloroform
- 1% ammonia
- Sodium hydroxide
- Glacial acetic acid
- Dilute HCl
- Concentrated H_2SO_4

4.1.3. Equipments & Instruments

Glasswares and plastic wares used for conducting experiment including test tubes and pipettes.

4.2. Methods

25g of stored dried powder was weighed in a weighing balance. The weighted powder is moved to a beaker and 250mL of distilled water is added to the beaker with constant stirring with a glass rod. The beaker is made air tight and kept at room temperature for 48 hours with occasional stirring. After 48 hours the extract is filtered with a cheesecloth. The aqueous extract is collected and stored for future use.

4.3. Phytochemical Screening Tests

4.3.1. Test for alkaloids

a) Mayer's test: To 1mL extract added 2mL of Mayer's reagent (Potassium Mercuric Iodide). Formation of a cream coloured precipitate indicated the presence of alkaloids.

b) Wagner's test: To 1mL extract added 2mL of Wagner's reagent (Iodine in potassium iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Draggendroff's test: To 1mL of extract added 1mL Dragendroff's reagent (solution of potassium bismuth iodide) was added. Formation of orange red precipitate indicates the presence of alkaloids.

4.3.2. Test for flavonoids

a) Lead acetate test: Extract was treated with few drops of lead acetate solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicated the presence of flavonoids.

b) Alkaline reagent test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicated the presence of flavonoids.

c) Treatment with Ammonia: The extract was treated with 1% ammonia, formation of yellow colour indicated the presence of flavonoids.

4.3.3. Test for phenolic compounds and tannins

a) Treatment with basic lead acetate: 1mL of the test solution was mixed with basic lead acetate solution and the formation of white precipitate indicated the presence of tannins and phenolic compounds.

b) Treatment with ferric chloride: To 1mL of extract, ferric chloride solution was added. Formation of a dark blue or greenish black coloured product confirmed the presence of phenolic compounds and tannins.

c) Treatment with potassium dichromate: Strong potassium dichromate solution was added to the test extract, yellow coloured precipitate confirmed the presence of tannin and phenolic compounds.

4.3.4. Test for glycosides

a) Legal's test: 1mL of the extract was treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicated the presence of cardiac glycosides.

b) 5mL of aqueous extract was mixed with 2mL of glacial acetic acid containing 1 drop ferric chloride. This mixture is carefully added to 1mL concentrated sulphuric acid (The concentrated sulphuric acid is seen underneath the mixture). Formation of a brown ring indicates the presence of glycosides.

4.3.5. Test for saponins

a) Froth test: 1mL of the extract was diluted to 20 mL with distilled water and was shaken in a graduated cylinder for 15 minutes. Formation of 1cm thick layer of foam indicates the presence of saponins.

b) Foam test: 0.5g of extract was shaken with 2mL of distilled water. If the foam produced persists for 10 minutes it indicates the presence of saponins.

4.3.6. Test for terpenoids

a) Salkowski test for terpenoids: 5mL of extract was added with 2mL of chloroform and 3mL of concentrated sulphuric acid was added along the sides of the test tube. Appearance of a reddish brown colouration at the interface indicates the presence of terpenoids.

4.3.7. Test for steroids

a) Salkowski test for steroids: 1mL of the steroids was treated with equal volume of chloroform and 3mL of concentrated sulphuric acid was added along the sides if the test tube. Upper layer turns red and lower layer turns yellow with green fluorescence indicate the presence of steroids (Yousaf st al., 2018)

4.4. Results & Discussion

The powdered flowers of *C. odorata* were extracted with water. The resultant cold extracted aqueous solution was subjected to screening of various phytochemical constituents. **Table.4.** shown below indicates the presence and absence of various phyto constituents in the aqueous extracts of *Chromolaena odorata* flower. It revealed the presence of alkaloids, flavonoids, phenols, glycosides, saponins and terpenes. The aqueous extract revealed the absence of steroids.

Table.4. Phyto constituents of the aqueous extract of *C. odorata* flower

Sl. No.	Name of Test	Results
1.	Test for alkaloids	+
2.	Test for flavonoids	+
3.	Test for phenolic compounds and tannins	+
4.	Test for glycosides	+
5.	Test for saponins	+
6.	Test for terpenoids	+
7.	Test for steroids	–

+ Indicate the Presence of Constituents; - Indicate the Absence of Constituents

Fig.4. Results of phytochemical screening of the aqueous extract of *Chromolaena odorata*

The secondary metabolites from plants, which are distinguished from primary metabolites such as nucleic acids, amino acids, carbohydrates, fats, etc., are extremely diverse. The plant chemical used for drug purposes are largely the secondary metabolites and are not directly involved in growth, development and reproduction of plant. These secondary metabolites can be classified into several groups according to their chemical classes as terpenoids, alkaloids, phenolics, etc. (Harborne, 1998).

Plants synthesise secondary metabolites (small organic molecules) that are not required for their normal growth or development but are essentially required for reproduction and defence mechanisms against bacteria, fungus, virus, vertebrates, etc. These products have a great potential to act as drugs. Many secondary metabolites are involved in the antagonistic relationship between plants and other organisms, but also in mutualistic ones (i.e., plants/pollinators, plants/disseminators, nitrogen-fixing plants/microorganisms, etc.). Secondary metabolites are the heterogeneous group of naturally occurring compounds, which have been used to treat various diseases. The biochemistry of medicines based on traditional natural products have made a tremendous contribution to public healthcare and has boosted the development of affordable medicines globally. Secondary metabolites have been investigated extensively since the 1850s. Their classification can be based on the chemical composition (containing nitrogen or not), chemical structure (e.g., having rings, containing a sugar), the biosynthetic pathway (e.g., phenylpropanoid, which produces tannins) or their solubility. They are divided into three large categories, namely alkaloids, terpenes, and phenolics. The greater part of plant derived compounds are phytochemicals, and secondary metabolites, which play a dominant role as antimicrobials and antivirals and are classified in many groups such as, alkaloids, phenolics, polyphenols, flavonoids, quinones, tannins, coumarins, terpenes, lectins and polypeptides, saponins, etc. (Anand et al., 2019).

Medicinal plants are the potent source of human health due to the presence of active phytochemical compounds that are responsible for its various pharmacological activities. On the basis of the results obtained, the present work concludes that the flowers of *Chromolaena odorata* are rich in phytochemical constituents and their medicinal value is due to these phytoconstituents. Antioxidant activity of the plant extract is often associated with the phenolic compounds present in them. They can react with active oxygen radicals, such as hydroxyl radicals, superoxide anion radicals and lipid peroxyl radicals and inhibit the lipid peroxidation at an early stage. Thus, bioactive compounds present in *Chromolaena odorata* makes them a potential candidate for various biochemical studies beneficial for humans and the economy.

5. Quantitative Determination of Secondary Metabolites in Aqueous Extract of *Chromolaena odorata* Flower

5.1. MATERIALS

5.1.1. Plant materials

Samples used in the study were flowers of *Chromolaena odorata* collected from Kollam, Kerala, India. The plants were collected during the months of January to March. Plant material was washed several times with tap water and once with distilled water and allowed to shade dry at room temperature for 3-7 days. Dried plant materials were powdered with the help of a blender and stored in an airtight container. This powder is used for quantitative estimation.

5.1.2. Chemicals

- Gallic acid
- Folin's Ciocalteu reagent (1:4 diluted)
- Sodium carbonate (75g in 100mL distilled water)
- Ascorbic acid
- Phosphomolybdenum reagent

a) 0.6M H_2SO4

b) 28mM sodium phosphate

c) 4mM ammonium molybdate

5.1.3. Equipments and Instruments

- Pipettes (2mL & 10mL)
- Water bath
- Colorimeter
- Weighing balance
- Standard flask
- Centrifuge
- Mortar and pestle
- Vortex mixer

5.2. METHODS

5.2.1. Preparation of aqueous flower extract

100mg of the dry, powdered flower is grinded with 10mL distilled water. It is then centrifuged at 2000rpm for 10 minutes. 1mL of the supernatant is pipetted out and made up to 5mL with distilled water .0.5ml of this solution is taken as test solution.

5.2.2. Determination of total phenolic content

The total phenolic content in the aqueous extract and water was determined by Folin-Ciocalteu method (Mc Donald et al., 2001). For the preparation of calibration curve 1mL aliquots of 8, 16, 24, 32 and 40µg/mL gallic acid solutions were mixed with 5mL Folin-Ciocalteu reagent and 4mL sodium carbonate. The tubes were allowed to stand at room temperature for 5 minutes. Absorbance of the sample was measured against blank at 650nm using a colorimeter. A calibration curve of gallic acid was prepared and the amount of total phenolic content was estimated.

5.2.3. Determination of total antioxidant activity

The antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure described by Prieto et al., (1991). The assay is based on the reduction of Mo(**VI)** to Mo(**V**) by the aqueous extract and subsequent formation of a green coloured phosphate/Mo(**V**) complex at acidic pH. A 0.5 mL extract was combined with 3mL of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95℃ for 90 minutes. Then the absorbance of the solution was measured at 690nm using a colorimeter against blank after cooling to room temperature. Distilled water in place of extract was used as blank. From the standard ascorbic acid curve the total antioxidant activity was calculated.

5.3. RESULTS AND DISCUSSION

5.3.1. Determination of total phenolic content

In acid medium, the phenolic compounds react with sodium tungstate and sodium molybdate in the Folin's reagent to form blue coloured product. The aqueous flower extract of *Chromolaena odorata* showed the presence of a considerable amount of total phenolic content shown in **Table.5.** The value was experimentally determined from the standard gallic acid curve shown in **Fig.5.** Phenolic compounds possess redox properties thus constituting the antioxidant nature of the aqueous extract.

Fig.5. Standard gallic acid curve

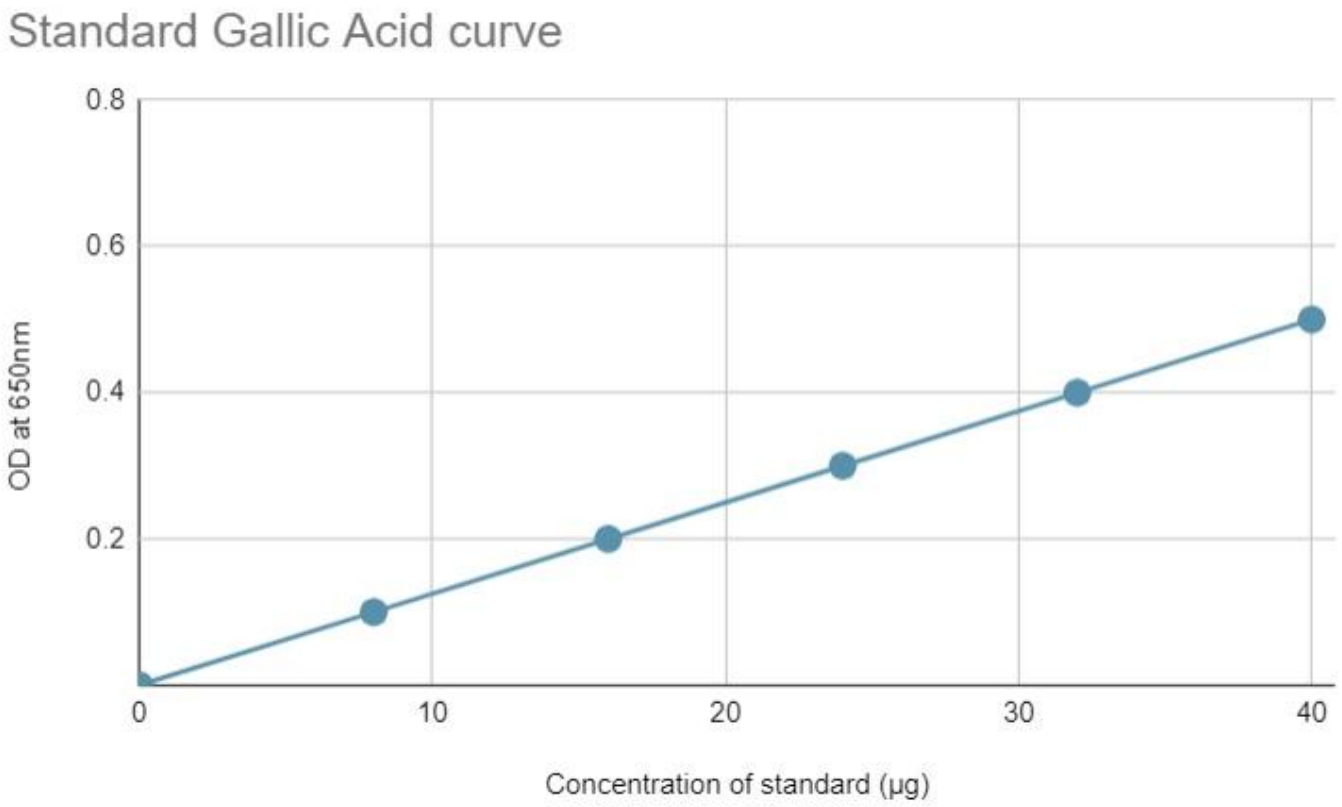

Table.5. Table showing Total phenolic content

C. odorata flower	Total Phenolic content (mg GAE/g dry extract)
Aqueous extract	47.99 ± 0.87

Values are mean ± SD (n=3)

The total phenolic content in the aqueous flower extract of *Chromolaena odorata* = 47.99 ± 0.87

5.3.2. Determination of total antioxidant activity

The aqueous flower extract of *Chromolaena odorata* exhibited high antioxidant activity shown in **Table.6.** The hydroxyl groups in the aqueous flower extract shows free radical scavenging activity. This property of phytochemicals is responsible for the antioxidant activity of the flower extract. The total antioxidant activity was experimentally calculated from the standard ascorbic acid curve shown in **Fig.6.**

Fig.6. Standard ascorbic acid curve

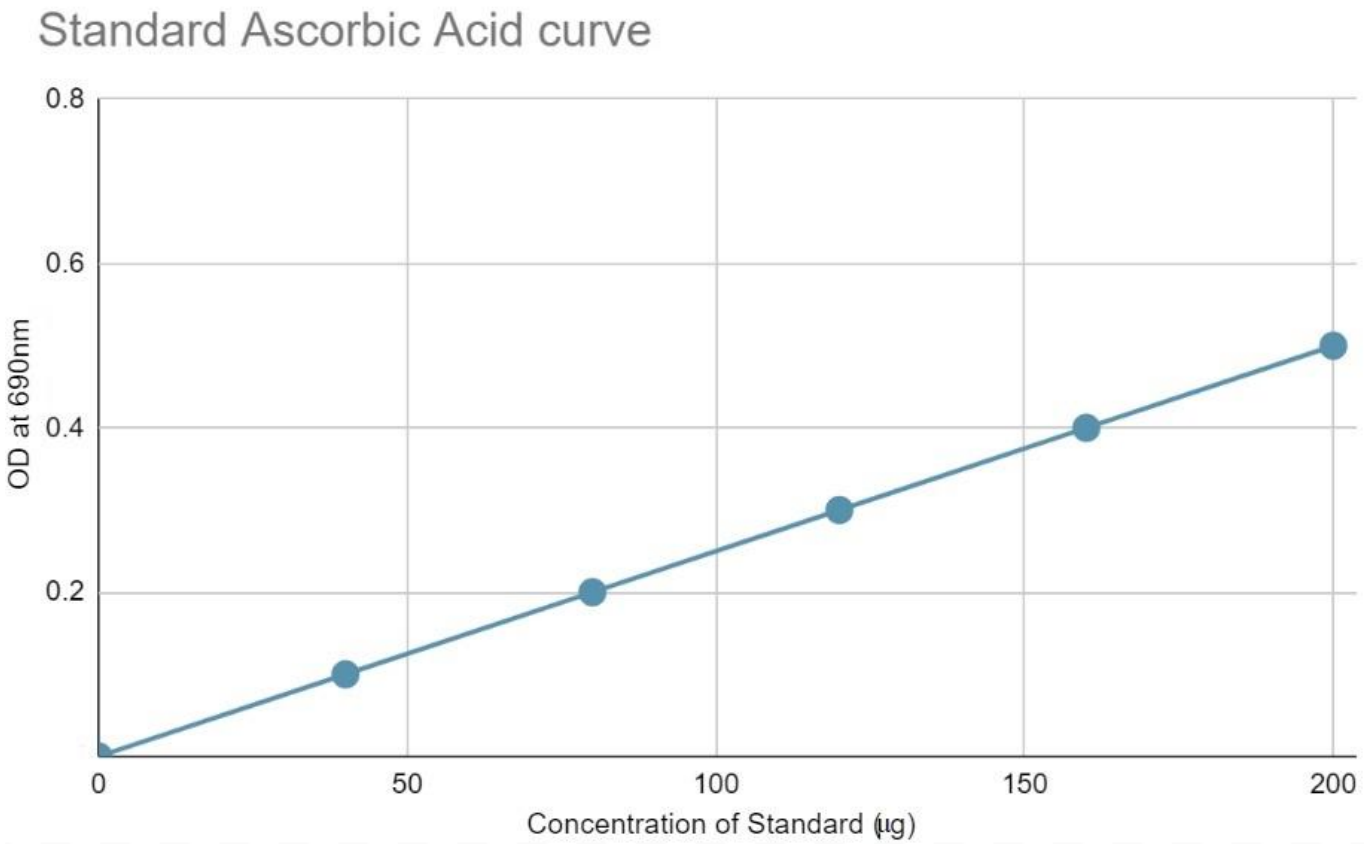

Table.6. Table showing Total antioxidant activity

C. odorata flower	Total Antioxidant activity (mg AAE/g dry extract)
Aqueous extract	130.66 ± 8.21

Values are mean ± SD (n=3)

The total antioxidant activity of the aqueous flower extract of *Chromolaena odorata* = 130.66 ± 8.21

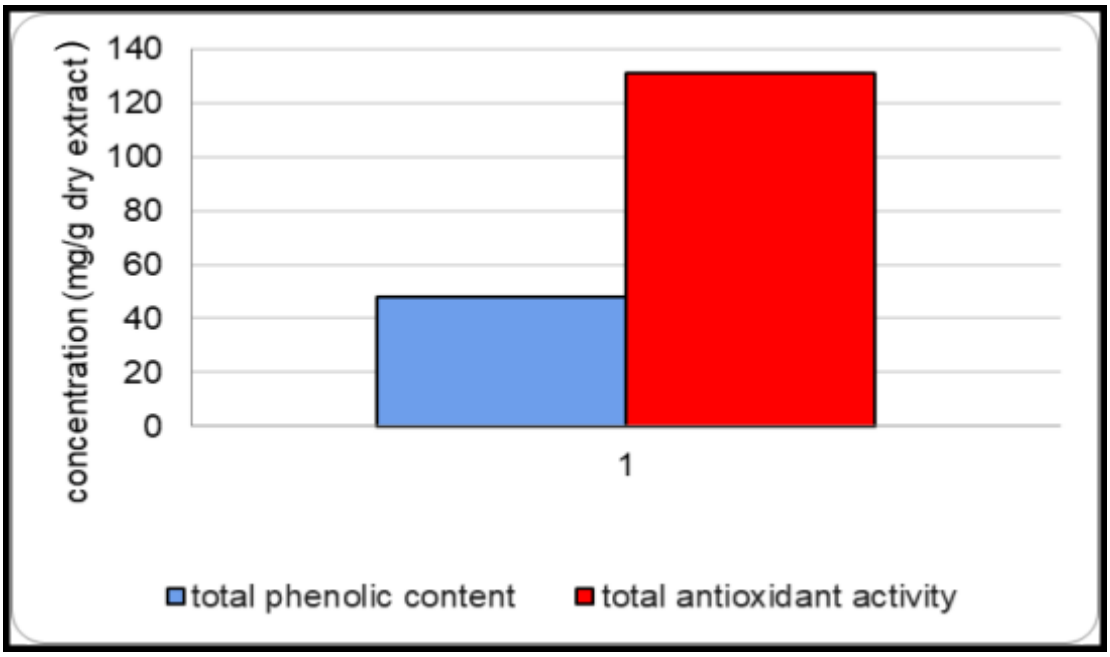

Fig.7. Diagrammatic representation of Total phenolic content & antioxidant activity

6. SUMMARY AND CONCLUSION

Chromolaena odorata L. belongs to the Asteraceae family (sunflower family) is an important and serious perennial herb in the world, while this weed also acts as a medicinal plant. Several parts of this plant are widely used to treat wound, burns, skin infections as well as to possess anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial, and antioxidant properties. Siam weed is one of the common names of *Chromolaena odorata* L. grown as medical herbs and ornamental plants. The medicinal values of *Chromolaena odorata* L. lie in their phytochemicals component, the dried leaf of Chromolaena odorata contained flavonoid aglycones (flavanones, flavonols, flavones) including acacetin, chalcones, eupatilin, luteolin, naringenin, kaempferol, quercetin, quercetagetin, and sinensetin, terpenes and terpenoids, essential oils, and other phenolic compounds, which produce define physiological action in our body.

To understand its specific role as nature's gift for healing wounds and its contribution to affordable healthcare, this plant must be scientifically assessed based on the available literature.Therefore, this phytochemical screening provides a brief insight about the various secondary metabolites in the aqueous extract of the flower of *C. odorata.* The invasive nature of this weed is used to control other more harmful weeds to crops. Moreover, *C. odorata* increases the mineral content of the soil thus showing potential sector. Feeding Siam weed to laboratory test animals like rabbits showed a similar result to that of a concentrated feed. Therefore, *C. odorata* can be a viable food or nutrient supplement for rearing rabbits and rodents.

7. REFERENCE

1. Ahmad, M. and M.N. Nabi., (1969), Chemical Investigations on the Leaves of *Eupatorium odoratum*. **Science Research**, 6: 37-41

2. Akinmoladun, A. C., Ibukun, E. O., Dan-Olge, I. A., (2007), Phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata*. **Scientific Research and Essay**, Vol 2 (6): 191-194

3. Ambika, S.R. and Jayachandra., (1980), Influence of light on seed germination in *Eupatorium odoratum* L. **Indian Forester**, 106: 637-640

4. Anand, U., Jacobo-Herrera N., Altemimi, A., Lakhssassi, N., (2019), A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. **Metabolites**. 9(11):258

5. Angitha Ajay., Rupesh Kumar, M., Shamal Badhusha, PA., Blainy Baby., Abhishek, K., Sanjay, K., Gowda,, B.Ramesh., (2021), Pharmacological Importance of *Chromolaena odorata*: a review.**International Journal of Pharmaceutics and Drug Analysis**, Vol(9): 8-11

6. Anyanwu, S., Eno, EO., Inyang. IJ., Asemota, EA., Okpokam, DC., Obioma, OO., Agu, VO., Emeribe, AU., (2018), Evaluation of Hepatotoxicity Associated With Ethanolic Extract of *Chromolaena odorata* in Wistar Rat Model. **IOSR Journal of Dental Medicine and Science**, 17(7):1-8

7. Apori, S.O., Long, R.J., Castro, F. B. and Rskov, E.R., (2000), Chemical composition and nutritive value of leaves and stems of *Chromolaena odorata*. **Grass and Forage Science**, 55:77-81

8. Apori., Long., Castro and Orskov., (2001), Chemical composition and nutritive value of leaves and stems of tropical weed *Chromolaena odorata*. **Phytochemistry**, Vol 55(1): 77-81

9. Athnasiadou, S., Kyriazakis, I., Jackson, F., Coop, RL., (2001), Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: In vitro and in vivo studies. **Veterinary Parasitology**, 99:205-219

10. Bamba, D., Bessiere, JM., Marion, C., Pelissier, Y., Fourate, I., (1993), Essential oil of *Eupatorium odoratum*. **Planta Medica**, 59: 184-185

11. Bamikole, M.A., Ikhatua, U.J. and Osemwenkhee, A.E., (2004), Converting bush to meat: Case of *Chromolaena odorata* feeding to rabbits. **Pakistan Journal of Nutrition,** 3: 258 261

12. Barliana, M. I., Suradji, E. W., Abdulah, R., Diantini, A., Hatabu, T., Nakajima-Shimada, J., Koyama, H., (2014), Antiplasmodial properties of kaempferol-3-0-rhamnoside isolated from the leaves of *Schima wallichii* against chloroquine-resistant *Plasmodium falciparum*. **Biomedical Reports,** 2: 579-583

13. Baruah, R. N., Sharma, R. P., Thyagarajan, G. and Werner, H. (1978), Flavonoid of *Chromolaena odorata*. **Phytochemistry**, Vol 17: 1807-1808

14. Baskin, Y. (2002), A Plague of Rats and Rubbervines: The Growing Threat of Species Invasions. **Island Press, Washington, DC, USA**, 1-50

15. Bate-Smith, EC., (1962), The phenolic constituents of plants and their taxonomic significance 1. Dicotyledons. **Journal of Linnean Society Botany,** 58:95-173

16. Bennett, F.D. and Rao, V.P., (1968), Distribution of an introduced weed *Eupatorium odoratum* L. in Asia and Africa and possibilities of its biological control. **9th Commonwealth Forestry Conference, Proceedings. PANS(C)** 14: 277-281

17. Bhargava, D., Mondal, CK., Shivapuri, JN., Mondal, S., Kar, S., (2013), Antioxidant properties of the leaves of *Chromolaena odorata* Linn. **Journal of Institute of Medicine Nepal**, 35(1):53-7

18. Biller, A., Boppré, M., Witte, L. and Hertmann, T., (1994) Pyrrolizidine alkaloids in *Chromolaena odorata*. chemical and chemoecological aspects. **Phytochemistry**, Vol 35(3): 615-61

19. Boucher, H.W., Ambrose, P.G., Chambers, H.F., Ebright, R.H., Jezek, A., Murray, B.E., Newland, J.G., Ostrowsky, B., Rex, J.H., (2017), White paper: Developing antimicrobial drugs for resistant pathogens, narrow-spectrum indications, and unmet needs. **Journal of Infectious Disease**, 216, 228-236

20. Bukirwa, H., Orton, L., (2005), Artesunate plus mefloquine versus mefloquine for treating uncomplicated malaria. **Cochrane Database of Systematic Reviews,** CD004531. 10.1002/14651858.CD004531.pub2

21. Bunyapraphatsara, N. and Chokechajiaroenprom, O., (2000), Faculty of Pharmacy, Mahidol University and National Center for Genetic Engineering and Biotechnology, Bangkok. **Thai Medicinal Plants**, Vol 4: 622-626

22. Chandrasekaram, S., Swamy, P.S., (2002), Biomass, litterfall and aboveground net primary productivity of herbaceous communities in varied ecosystems at Kodayar in the western ghats of Tamil Nadu. **Agriculture Ecosystems & Environment,** 88: 61-71

23. Chen, S., Song, J., Sun, C., Xu, J., Zhu, Y., Verpoorte, R., Fan, T.P., (2015), Herbal genomics: Examining the biology of traditional medicines. **Science**, 347, S27-S29

24. Collins, LA., Franzblau, SG., (1997), Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. **Antimicrobial Agents and Chemotherapy**, 41: 1004-1009

25. Coop, RL., Holmes, PH., (1996), Nutrition and parasite interaction. **International Journal of Parasitology**, 26:951-962

26. Dhar, R., Kimseng, R., Chokchaisiri, R., Hiransai, P., Utaipan, T., Suksamrarn, A., Chunglok, W., (2018), 2',4 Dihydroxy-3',4',6'-trimethoxy chalcone from *Chromolaena odorata* possesses anti inflammatory effects via inhibition of NF-kB and p38 MAPK in lipopolysaccharide-activated RAW 264.7 macrophages. **Immunopharmacology and Immunotoxicology**, 40(1):43-51

27. Dieneba., Bamba., Besserie, J., Marion, C., Pelissiere, Y. and Fouraste, I., (1992), Essential oil of *Eupatorium Odoratum*. Planta Med. Vol 59

28. Gamenara, DE., Pandolfi., Saldana, J., Dominguez, L., Martinez, MM., Seoane, G., (2001), Nematocidal activity of natural polyphenols from bryophytes and their derivatives. **Arzneimittelforschung**, 51:506-510.

29. Gareeb, M., Pammenter, N.W., Bower, J.P. and Zachariades, C., (2004), Determining optimal growth conditions for the South African biotype of *Chromolaena odorata*. In: Day, M.D. and McFadyen, R.E. (Editors) Chromolaena in the Asia-Pacific Region. ACIAR Technical Report No. 55. **Australian Centre for International Agricultural Research**, Canberra: 1-18

30. Garry, R. (1963), The changing fortunes and future of pepper growing in Cambodia. **Journal of Tropical Geography** 17: 133-142

31. Hanh, TTH., Hang, DTT., Van Minh, C., Dat., NT., (2011), Anti-inflammatory effects of fatty acids isolated from *Chromolaena odorata*. **Asian Pacific Journal of Tropical Medicine**, 4(10):760-3

32. Hanphanphoom, S., Krajangsang, S., (2016), Antimicrobial Activity of *Chromolaena odorata* Extracts against Bacterial Human Skin Infections. **Modern Applied Science**, 10(2):159

33. Harborne, J, B., (1998), Phytochemical methods: A guide to modern techniques of plant analysis, 3rd edition. **Chapman and Hall, London**

34. Heiss, EH., Tran,bTV., Zimmermann, K., Schwaiger, S., Vouk, C., Mayerhofer, B., Malain er C., Atanasov, AG , Stuppner, H and Dirsch, VM., (2014), Identification of chromomoric acid C-1 as an Nrf2 activator in *Chromolaena odorata*. **Journal of Natural Products**, 77:503-508. View Article: Google Scholar: PubMed/NCB

35. Holm, GLR., Plucknet, DL., Pancho, JV., Herberger, JP., (ed.), (1977), *Chromolaena odorata*. In The World's Worst Weeds: Distribution and Biology. **The University Press of Hawaii**, 212-216

36. Howard, R.A., (1989), Flora of the Lesser Antilles, Leeward and Windward Islands. Vol. 6, **Arnold Arboretum**, Harvard University, Jamaica Plain, MA, 1-658

37. Irobi, O. N., (1997), Antibiotic preparation of ethanolic extract of *Chromolaena odorata* (Asteraceae). **International Journal of Pharmacognosy**, Vol 35(2); 111-115

38. Iwu, M. M., (1993), Handbook of African Medicinal Plants. **CRC Press**, Ann Arbor, 181-182

39. Jones, N.C.L., (1954), Numinbah horse sickness. Institute of Inspectors of Stock of New South Wales Year Book. **Government Printer, Sydney**, 1-84

40. Joy, P.J., Lyla, K.R. and Abraham, C.C., (1979), Preliminary studies on the aphid pests of *Eupatorium odoratum* Linn., an important weed in plantations of Kerala. **2nd Annual Symposium on Plantation Crops Proceedings**, Ooty, 272-275

41. Kanase, VA., Shaikh, SA., (2018), A Pharmacognostic and Pharmacological Review on *Chromolaena odorata* (L.) RM King & H. Rob.(Siam weed). **Asian Journal of Pharmaceutical and Clinical Research**, 11(10):34-8

42. Kumalasari, N. R., Abdullah, L. and Jayadi, S., (2005), Pengaruh pemberian mulsa *Chromolaena odorata* (L.) Kings and Robins pada kandungan mineral P dan N tanah latosol dan produktivitas hijauan jagung (*Zea mays* L.). **Journal of Animal Science and Technology,** Vol. 28 (1)

43. Kumar Shakya,A., Arvind Kumar Shakya, C., (2016), Medicinal plants: Future source of new drugs. **International Journal of Herbal Medicine**, 4(4):59-64

44. King, R. M. and Robinson, H., (1970), Studies in the Eupatoriae (Compositae). ***The Genus Chromolaena Phytologia*** *20: 196-209*

45. Lahlou,M., (2002), Potential of *Origanum compactum* as a cercaricide in Morocco. **Annals of Tropical Medicine and Parasitology**, 96:587 593

46. Le, TT., (1995), The use of Eupolin prepared from *Eupatorium odoratum* to treat soft tissue wounds. **The 5 European tissue repair society annual meeting**, Padova, Italy (Abst.30)

47. Lewis, DA and Hanson, PJ., (1991), Antiulcer drugs of plant origin. **Progress in Medical Chemistry**, 8:21 0-229

48. Liogier, H. A., (1997) Descriptive flora of Puerto Rico and adjacent Islands. **Editorial de la Universidad de Puerto Rico**, San Juan, Vol(5): 436

49. Ling, S.K., M.A, Nor Azah., M, Mastura., M.G.H, Khoo., S, Saidatul Husni., M, Salbiah., A, Abdul Rashid., M.O, Mazura., S, Vimala., B.K, Ong and A.B, Siti Asha., (2007a) Extraction and simultaneous detection of flavonoids in the leaves of *Chromolaena odorata* by RP-HPLC with DAD. In: Nik Zanariah, N. M.(Eds.) Highlights of FRIM's IRPA Projects 2005. Identifying Potential Commercial Collaborations Project Evaluation Meeting. 14-15 Dec 2005, FRIM

50. Ling, S. K., Mazura Md Oisar and Salbiah Man., (2007b), Platelet-Activating Factor (PAF) Receptor Binding Antagonist Activity of the Methanol Extracts and Isolated Flavonoids from *Chromolaena odorata* (L.) King and Robinson. **Biological and Pharmaceutical Bulletin**, 30 (6): 1150-1152

51. Litzenberger, S. and Lip, Ho Tong., (1961), Utilizing *Eupatorium odoratum* L. to improve crop yields in Cambodia. **Agronomy Journal** 53: 321-324

52. Madhuri, S., Pandey, G., (2008), Some dietary agricultural plants with anticancer properties. **Archives**, 8(1):13-6

53. Marianne, D. L. P., Y. S.l, Elin., F. K, Neng., and N, Rosnani., (2014), Antidiabetic Activity of Leaves Ethanol Extract *Chromolaena odorata* (L.) R.M. King on Induced Male Mice With Alloxan Monohydrate. **Jurnal Natural**, 14(1):1-4

54. Martin, RJ., (1997), Mode of action of Anthelmintic drugs. **Veterinary Journal**, 154:11-34

55. Martin, P., (1997), Wound healing-aiming for perfect skin regeneration. **Journal of Science**, 276:75 -81. View Article: Google Scholar: PubMed/NCBI

56. Mayela Nkouka, S.H.J., Ktéssé, D.S., Elion Itou, R.D.G., Etou Ossibl, A.W., Abena A.A., (2019), Evaluation of antidiarrheal effect of the aqueous extract of the leaves of *Chromolaena odorata* L (King and Robinson). **Journal of Drug Delivery and Therapeutics**, 9(2):367-371 http://dx.doi.org/10.22270/jddtv912.2392

57. M'Boob, S.S., (1991), Preliminary results of a survey and assessment of *Chromolaena odorata* (Siam weed) in Africa. **Biotropica Special** Pub. 44: 51-55

58. Mc Donald, S., Prenzler, P D., Autovich, M & Robards, K., (2001); Phenols content and antioxidant activity of olive extracts; **Journal of Chemistry**, 73,73-84

59. McFadyen, R.E.C., (1989), Siam weed: A new threat to Australia's north. **Plant Protection Quarterly,** 4: 3-7

60. McFadyen, R. and Skaratt, B., (1996), Potentian Distribution of *Chromolaena odorata* (siam weed) in Australia, Africa and Oceania. **Agriculture, Ecosystems and Environment**, Vol 59: 89-96

61. McFadyen, R.C., (2003), *Chromolaena* in Southeast Asia and the Pacific. In: Costa, H.D., Piggin, C., da Cruz, C.J. and Fox, J.J. (Editors) Agriculture: New Directions for a New Nation East Timor (Timor-l' Este) ACIAR. **Australian Centre for International Agricultural Research**, Canberra. Proceedings No. 113: 130-134

62. Morton, JF., (1981), Atlas of Medicinal Plants of Middle America, Vol. II. **Charles C. Thomas Publisher,** Springfield, Illinois, USA

63. Muhammad, Z. and A.M. Mustafa., (1994), Traditional Malay Medicinal Plants. **Fajar Bakti S/Bhd**

64. Muniappan, R. and Viraktamath, C.A., (1986), Insects and mites associated with *Chromolaena odorata*. **Entomon** 11(4): 285-287

65. Muniappan, R and Jesse Bamba., (2000), Biological control of *Chromolaena odorata*: Successes and Failures. **Proceedings of the X International Symposium on Biological Control of Weeds** (4-14 July 1999) Montana State University, USA, 81-85

66. Muricken Deepa, G., Joy Jofeena., (2015), Analysis of Phytochemicals from *Eupatorium odoratum* Flower. **International Journal of Ayurveda and Pharma Research**, 3(7):1-5

67. Nisit, P ., Saisunee, L. B., B. J., Bconsom, L., (2005), Chemical Constituents and Biological Activities of *Chromolaena odorata*. **Chiang Mai Journal of Science**, 32: 139-148

68. Norgrove, L., Hauser, S., Weise, S.F., (2000), Response of *Chromolaena odorata* to timber tree densities in an agrosilvicultural system in Cameroon; aboveground residue decomposition and nutrient release. **Agriculture Ecosystems & Environment,** 81: 191 207

69. Nudo, LP., Catap, ES.,(2012), Anti-immunosuppressive effects of chromolaena odorata (Lf.) king & robinson (asteraceae) leaf extract in cyclophosphamide-injected Balb/C mice. **Philippine Journal of Science**, 141(1):35-43

70. Odunbaku, O. A. and Ilusanya, O. A., (2008), Antibacterial activity of the Ethanolic and methanolic leaf extracts of some tropical plants on some human pathogenic microbes. **Research Journal of Agriculture and Biological Science**, Vol 4(5): 373-376

71. Offor, U. S. and Akonye, L. A., (2006), Amendment fo crude oil contaminated soil with sawdust leaves for optimum plant protection. **African Journal of Biotechnology**, Vol 5 (9): 770-774

72. Ogbonnia, SO, Mhaka, GO., Anyika, EN., Osegbo, OM., Igbokwe, NH.,(2010), Evaluation of acute toxicity and subchronic toxicity of in mice and hydroethanolic extract of *Chromolaena odorata*(L.) King and Robinson (Fam. Asteraceae) in rats. **Agriculture and Biology Journal of North America**, 1(5):859-865

73. Omokhua, AG., McGaw, LJ., Finnie, JF., Van Staden, J., (2016), *Chromolaena odorata* (L.) R.M. King & H. Rob. (Asteraceae) in sub-Saharan Africa: A synthesis and review of its medicinal potential. **Journal of Ethnopharmacology**, 183:112-22

74. Omonije, OO., Saidu l, AN., Muhammad, HL., (2019), Anti-diabetic activities of *Chromolaena odorata* methanol root extract and its attenuation effect on diabetic induced hepatorenal impairments in rats. **Clinical Phytoscience**, 5(1):23.

75. Onoja, S., Nweze, E., Ezeja, M., Omeh, Y., Obi, C., (2016), Evaluation of the Antioxidant and Analgesic Activities of Hydromethanolic Extract of *Chromolaena odorata* Leaf. **British Journal of Pharmaceutical Research**, 10(5):1-7

76. Ooi, P.A.C., Sim, C.H. and Tay, E.B., (1988), Status of the arctiid moth introduced to control Siam weed in Sabah, Malaysia, **Planter Kuala Lumpur**, 64: 298-304

77. Oweyele, B. V., Oguntoye, S. O., Dare, K., Ogunbiyi B. A., Aruboula E. A. and Soladye, A. O., (2008), Analgesic, anti-inflammatory and antipyretic activities from flavonoid fraction of *Chromolaena odorata*. **Journal of Medicinal Plant Research,** Vol 2(9): 219-225

78. Pancho, J.V. and Plucknett, D.L., (1971), *Chromolaena odorata* (L.) R.M. King and H.R. Robinson- A new record of a noxious weed in the Philippines. **Philippine Journal of Animal Science,** 8: 143-149

79. Panda, D., Dash, S.K. and Dash, G.K., (2010), Qualitative phytochemical analysis and investigation of anthelmintic and wound healing potentials of various extracts of *Chromolaena odorata* linn. collected from the locality of mohuda village, berhampur (south orissa). **International Journal of Pharmaceutical Sciences Review and Research**, 1(2): 122-126

80. Pandurangan, A., Kavita, R., Apoorva, S., (2015), Evaluation of antimicrobial and anthelmentic activity of leaves of *Chromolaena odorata*. **International Bulletin of Drug Research**, 5:64-71

81. Paul, TS., Das, BB., Ingale, SP., Killedar, N., Apte, KG., (2018), Oral intake of polyphenols of *Chromolaena odorata*: A perspective in peptic ulcer, thrombocytopenia, and heparin-induced bleeding diathesis in rodent model. Pharmacognosy Research, 10(4):426-31

82. Pérez-Amador, M. C., Muñoz Ocotero, V., Ibarra Balcazar, R., & García Jiménez, F., (2010), Phytochemical and pharmacological studies on *Mikania micrantha* H.B.K. (Asteraceae). **International Journal of Experimental Botany**, 79, 77-80

83. Phan TT, M.D., Margaret A. Hughes, Ph.D., George W. Cherry, D.Phil., Trung T. Le, D.Sc., and Hung M. Pham, D.Sc., (1996), An Aqueous Extract of the Leaves of *Chromolaena odorata* (Formerly *Eupatorium odoratum*) (Eupolin) Inhibits Hydrated Collagen Lattice Contraction by

Normal Human Dermal Fibroblasts. THE JOURNAL OF ALTERNATIVE AND COMPLEMENTARY MEDICINE Volume 2, Number 3: 335-343

84. Phan, TT., Wang, L., See, P., Grayer, RJ., Chan, SY and Lee, ST., (2001), Phenolic compounds of *Chromolaena odorata* protect cultured skin cells from oxidative damage: Implication for cutaneous wound healing. **Biological and Pharmaceutical Bulletin**, 24:1373-1379. View Article: Google Scholar: PubMed/NCBI

85. Phan, T.T., S, Patrick., Lee, S.T., and Cham, S.Y., (2001a), Anti-oxidant Effects of the Extracts from the Leaves of *Chromolaena odorata* on Human Dermal Fibroblasts and Epidermal Keratinocytes against Hydrogen Peroxide and Hypoxantinine-xantinine Oxidase. **Elsevier Science**, 319-327

86. Phan, T.T., Whan Lingzhi., S, Patrick., Grayer, R.J., Chan, S.Y. and Lee, S. T., (2001b), Phenolic Compound of *Chromolaena odorata* Protect Cultured Skin Cells from Oxidative Damage: Implication for Cutaneous Wound Healing. **Biological and Pharmaceutical Bulletin**, 24 (12): 1373-1379

87. Pisutthanan, N., Liawruangrath, B., Liawraungrath, S. and Bremmer, B., (2006), A new flavonoid from *Chromolaena odorata*. **Natural Product Research**, 20(13):1192-1198

88. Prieto, P., Pineda, M & Aguilar, M., (1999), Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. **Analytical Biochemistry**, 269(2): 337-341

89. Pye, C.R., Bertin, M.J., Lokey, R.S., Gerwick,W.H., Linington, R.G., (2017), Retrospective analysis of natural products provides insights for future discovery trends. **Proceedings of the National Academy of Sciences of the USA**, 114: 5601-5606

90. Rahman, A., Alam, MS., Khan, S., Ahmed, F., Islam, A & Rahman, M., (2008), Taxonomic Studies on the Family Asteraceae (Compositae) of the Rajshahi Division. **Research Journal of Agriculture and Biological Sciences**, 4: 134-140

91. Raizada, M.B., (1976), Supplement to Duthie's Flora of the Upper Gangetic Plain and of Adjacent Siwalik and Sub Himalayan Tracts. **Bishen Singh Mahendra Pal Singh**, Dehradun, 1-358

92. Ramani, N. and Haq, M.A., (1983), Oribatid mites (Acari) associated with *Eupatorium odoratum*. **Indian Journal of Acarology**, 8: 95-99

93. Rauf, A., Jehan, N., Ahmad, Z., Mubarak, MS., (2017), Analgesic potential of extracts and derived natural products from medicinal plants in Maldonado, C. (Ed.). Pain Relief From Analgesics to Alternative Therapies. **IntechOpen**, 24:339-351

94. Riddoch, J.C., Fasehun, F.E., Riddoch, B. and Lipido, D.O., (1991), Photosynthesis and successional status of seedlings in a tropical semi-deciduous rain forest in Nigeria. **Journal of Ecology**, 79: 491-503

95. Robinson, M.M. & Zhang, X., (2011), The World Medicines Situation 2011, Traditional Medicines: Global Situation, Issues and Challenges; World Health Organization: Geneva, Switzerland, 2011; 1-4

96. Roder, W., Phengchanh, S., Keoboualapha, B. and Mani phone, S., (1995), *C. odorata* in slash and burn rice systems of northern Laos. **Agroforestry Systems,** 31: 79 92

97. Saleem, M and Aftab, A., (1999), *Tephrosia purpurea* ameliorates benzoyl peroxide induced cutaneous toxicity in mice: Diminution of oxidative stress. **Pharmacy and Pharmacology Communications**, 5:455-461. View Article: Google Scholar

98. Shakya, AK., (2016), Medicinal plants: Future source of new drugs. **International Journal of Herbal Medicine**, 10: 53-64

99. Sirinthipaporn, A., Jiraungkoorskul, W., (2017), Wound healing property review of Siam weed, *Chromolaena odorata*. **Pharmacognosy reviews**, 11(21):35-38

100. Soogarun, S., Wiwanitkit, V. and Suwansaksri, J., (2005), Effect of *Chromolaena odorata* on prothrombin time and activated partial thromboplastin time. **HAEMA,** 8: 1-149

101. Srinivasa Rao, K., Chaudhury, PK and Pradhan, A., (2010), Evaluation of anti-oxidant activities and total phenolic content of *Chromolaena odorata*. **Food and Chemical Toxicology,** 48:729-732. View Article : Google Scholar: PubMed/NCBI

102. Subroto, M. A., (2006), Herbal Concoctions for Diabetes Melitus. Jakarta. **Penebar Swadaya**

103. Suksamram, A., Chotipong, A., Tananit S., Boongird S., Timsuksai P., Vimuttipong S. and Chuaynugul A., (2004), Antimycobacterial Activity and Cytotoxicity of Flavonoids from the Flowers of *Chromolaena odorata*. **Archives of Pharmacal Research**, Vol 27(5): 507-511

104. Sumitra, M., Manikandan, P and Suguna, L., (2005), Efficacy of Butea monosperma on dermal wound healing in rats. **Journal of Biochemistry and Cell Biology**, 37:566-573. View Article : Google Scholar

105. Talapatra, S. K., Bhar, D. S. and Talapatra, B., (1974), Flavonoid and Terpenoid Constituents of *Eupatorium odoratum*. **Phytochemistry**, 13: 284-285

106. Thompson, DP & Geary, TG., (1995), The structure and function of helminth surfaces. In: Biochemistry and Molecular Biology of Parasites (J. J. Marr, Ed.), 1st ed. **Academic Press**, New York. 1995,203-232

107. Torrenegra, RD and Rodríguez, OE., (2011), Chemical and biological activity of leaf extracts of *Chromolaena leivensis*. **Natural Product Communications**, 6: 947-950. PubMed/NC BI

108. Udayaprakash, NK., Sriraman, V., Devika, V., Peneena, V., Sripriya, N., Bhuvaneswari, S., Sampathkumar, B., (2019), Comparative studies on phytochemistry, antioxidant and antibacterial activity of direct and sequential extracts of *Chromolaena odorata* leaves. **International Journal of Research in Pharmaceutical Sciences**, 10(2): 914-921

109. Ukpai, O. M. and Amaechi, E. C., (2012), Evaluation of in vivo antimalarial activity of the ethanolic leaf extracts of *Chromolaena odorata* and *Cymbopogon citratus* in mice.**Nigerian Journal of Biotechnology**, Vol. 24: 27-34

110. Vaisakh, MN & Pandey, A., (2012), The invasive weed with healing properties: A review on chromolaena odorata. **International Journal of Pharmaceutical Science**, 3(1):80-3

111. Velasco-Alinsug, M.P., Rivero, G.C. and Quibuyen, A.O., (2005), Isolation of mercury-binding peptides in vegetative parts of *Chromolaena odorata*. **Zeitschrift für Naturforschung [C]**, 60 (3-4): 252-259

112. Vijayaraghavan,., Kavitha, S., A, Mohamed and R, Maruthi., (2013), Studies on Phytochemical Screening and Antioxidant Activity of *Chromolaena odorata* and *Annona squamosa*. **International Journal of Innovative Research in Science, Engineering and Technology**. 2(12): 7315-7321

113. Vijayaraghavan, K., Rajkumar, J., Bukhari, SNA., Al Sayed ,B & Seyed, MA., (2017), *Chromolaena odorata*: A neglected weed with a wide spectrum of pharmacological activities (Review). **Molecular Medicine Reports**, 15(3):1007-1016

114. Vijayaraghavan, K., Rajkumar, J & Seyed, MA., (2018), Phytochemical screening, free radical scavenging and antimicrobial potential of *Chromolaena odorata* leaf extracts against pathogenic bacterium in wound infections-a multispectrum perspective. **Biocatalysis and Agricultural Biotechnology**, 15:103-112

115. Waller, PJ., Bernes, G., Thomsborg, SM., Sukur, A., Richter, SH., Ingebrigtsen, K., Hoglund, J., (2001), Plants as deworming agents of livestock in Nordic countries: historical perspective, popular beliefs and prospects for the future. **Acta Veterinaria Scandinavica**, 42:31-44

116. Wang, Y., Waghorn, GC., Douglas, GB., Barry, TN., Wilson, GF., (1994), The effect of condensed tannins in *Lotus corniculatus* upon nutrient metabolism and upon body and wool growth in grazing sheep. **Proceedings of the New Zealand Society of Animal Production**, 54:219-222

117. Wang, L., Waltenberger, B., Pferschy-Wenzig, EM., Blunder, M., Liu, X., Malainer, C et al., (2014), Natural product agonists of peroxisome proliferator-activated receptor gamma (PPARY): A review. **Biochemical Pharmacology**, 92(1): 73-89

118. Widowati, W., (2008), The Potential of Antioxidant as Antidiabetic. **Journal of Knowledge Management**. 7(2): 1-10

119. World Health Organization (2008): Geneva, Switzerland.**World Malaria Report**. http://apps.who.int/malaria/wmr2008/

120. World Health Organization (2016): **Malaria Facts sheet**

http://www.who.int/mediacentre/factsheets/fs094/en/

121. WHO Technical Report Series 916, (2003).Diet, Nutrition and the Prevention of Chronic Diseases,, **Report of a Joint WHO/FAO Expert Consultation**, World Health Organization, Geneva : 88

122. Zachariades, C et al., (2009), *Chromolaena odorata* (L.) King and Robinson (Asteraceae). In: Biological control of tropical weeds using Arthropods, In: Muniappan R, Reddy GVP, Raman A (Eds.). **Cambridge University Press**, UK

123. Zhang, ML., Irwin, D., Li, XN., Sauriol, F., Shi, XW., Wang, YF., Huo ,CH., Li, LG., Gu, YC and Shi, QW., (2012), PPAR-gamma agonist from *Chromolaena odorata*. **Journal of Natural Products**, 75:2076-2081. View Article: Google Scholar: PubMed/NCBI

124. Zhang, Y. J., Gan, R. Y., Li, S., Zhou, Y., Li, A. N., Xu, D. P & Li, H. B., (2015), Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. **Molecules** (Basel, Switzerland), 20(12): 21138-21156