

Evaluation of Wound Healing Activity of *Heliotropium indicum* L. leaves

BY

S GAYATHRI

(Reg. No. 17PZO004)

Thesis Submitted to the

Avinashilingam Institute for Home Science and Higher Education

for Women, Coimbatore – 641 043

In Partial Fulfillment of the Requirements for the Degree of

Master of Science in Zoology

April 2019

**Evaluation of Wound Healing Activity of *Heliotropium*
indicum L. leaves**

BY

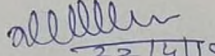
S GAYATHRI

(Reg. No. 17PZO004)

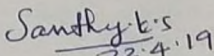
Thesis Submitted to the
Avinashilingam Institute for Home Science and Higher Education
for Women, Coimbatore – 641 043
In Partial Fulfillment of the Requirements for the Degree of

Master of Science in Zoology

April 2019


Signature of the

Head of the Department


Signature of the

Supervisor

ACKNOWLEDGEMENT

First and foremost I thank **Lord Almighty** for the manifold blessing showered upon me for completing my work successfully.

I am thankful to **Dr. T. S. K. Meeenakshisundaram**, M.A, M.Phil, Ph.D., and former Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing me with all the amenities for the smooth conduct of my thesis work.

I wish to express my profound sense of gratitude and sincere thanks to **Dr. P.R. Krishnakumar**, Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for all the amenities provided for the conduct of my research work.

I am extremely grateful to **Dr. Premavathi Vijayan**, M.Sc, M.Ed., Dip. Spl. Edn., M.Phil., Ph.D., Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for supporting me with all facilities needed for the completion of my thesis.

I wish to thank **Dr.Mrs.S.Kowsalya** M.Sc., M. Phil., Ph.D., Registrar, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing all the facilities needed for the completion of my thesis.

It is my pleasure to acknowledge the wholehearted support and encouragement extended to me by **Dr.P.R Padma**, M.Sc., Ph.D., Adv.Dip Dean, and School of Biosciences. Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing all the facilities needed for the completion of my thesis.

I am happy to express my hearty thanks to **Dr.Mrs.N.Krishnaveni**, M.Sc., B.Ed., Ph.D., NET Associate Professor and Head of the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, for her unflinching support and constant encouragement given throughout the tenure of my research.

I wish to place on record my indebtedness and profound gratitude to my Supervisor, **Dr. K. S. Santhy**, M.Sc., M. Phil., Ph.D., M.C.A., PGDBI, Associate Professor, Department of Zoology, Avinashilingam Institute for

Home Science and Higher Education for Women, Coimbatore, for her inspiring and ceaseless guidance, valuable suggestions and advice, patience, care, and kind help rendered throughout the study period and undoubtedly she will be the pathfinder for me.

I express my sincere thanks to **all the members of staff**, Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for their support.

I place a record of my heartfelt thanks to **my loving parents Late Mr.K.Senthil Nehru and Mrs.S.Annalakshmi, Sister N. Mehala devi and all my family members** for their love and support during my study period.

My thanks are also due to all those who have not been mentioned by name, but nevertheless, have been of tremendous help in their own ways.

S Gayathri

CONTENTS

CHAPTER NO.	TITLE	PAGE.NO
--------------------	--------------	----------------

List of Tables

List of Figures

List of Plates

List of Appendices

1	INTRODUCTION	1 - 7
2	REVIEW OF LITERATURE	8 - 24
	2.1 Natural plant product research	8
	2.2 Traditional medicine	10
	2.3 Phytochemicals and its significance	12
	2.4 <i>Heliotropium indicum</i> L. – an overview	15
	2.4.1 Ethnopharmacology	16
	2.4.2 Scientific classification of <i>Heliotropium indicum</i> L.	18
	2.4.3 Vernacular Names of <i>Heliotropium</i> <i>indicum</i> L.	18
	2.5 Therapeutic value – <i>Heliotropium indicum</i> L.	18
	2.6 Major chemical constituents and nutrients of <i>Heliotropium indicum</i> L.	21
	2.7 Wound Healing Activity of <i>Heliotropium</i> <i>indicum</i> L.	23
3	MATERIALS AND METHODS	25 -32
	3.1 Plant material and extract preparation	25
	3.2 Experimental animals	26
	3.2.1 Chemicals required	27
	3.2.2 Drug formulation	27
	3.2.3 Wound healing activity	27
	3.2.4 Excision wound model	28
	3.2.5 Epithelialisation period	28
	3.2.6 Wound contraction	28
	3.3 Statistical analysis	29

3.4	Antimicrobial activity	29
3.4.1	Test organisms	29
3.4.2	Methodology	29
3.4.3	Statistical analysis	30
3.5	Phytochemicals and its significance	30
3.5.1	Test for alkaloids	30
3.5.2	Test for flavonoids	30
3.5.3	Test for sterols	31
3.5.4	Test for phenols	31
3.5.5	Test for Saponins	31
3.5.6	Test for tannins	31
3.5.7	Test for proteins	31
4	RESULTS	33 - 45
4.1	Preliminary phytochemical screening of <i>Heliotropium indicum</i> L. leaves	33
4.2	Antimicrobial activity	35
4.2.1	Antibacterial activity of leaves extract of <i>Heliotropium indicum</i> L.	35
4.2.2	Antifungal activity of leaves extract of <i>Heliotropium indicum</i> L.	38
4.3	Wound healing activity	41
4.3.1	General observations	41
4.3.2	Wound contraction, complete epithelialization and complete healing	41
5	DISCUSSION	46 -49
6	SUMMARY AND CONCLUSION	50 - 51
7	REFERENCES	52 - 64
8	APPENDICES	65

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
-----------	-------	----------

I	Preliminary phytochemical analysis of <i>Heliotropium indicum</i> L. leaves	34
II	Antibacterial activity of the leaves extracts of <i>Heliotropium indicum</i> L against the bacterial isolates	36
III	Antifungal activity of the leaves extracts of <i>Heliotropium indicum</i> L against the fungal isolates	39
IV	Effect of EEHI on percent wound contraction and epithelialization period	41
V	Effect of ethanol extract on <i>Heliotropium indium</i> L. on epithelialisation period	44

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO.
------------	-------	----------

I	<i>Heliotropium indicum</i> L plant	25
II	<i>Heliotropium indicum</i> L leaves	
	Powder	26
III	Experimental animals (Wistar albino Rats)	26
IV	Antibacterial activity of <i>Heliotropium indicum</i> L.	
	leaves extracts	37
V	Antifungal Activity of <i>Heliotropium indicum</i> L.	
	leaves extracts	40
VI	Effect of EEHI on wound contraction	42
VII	Effect of EEHI on wound contraction	
	(Percentage)	43
VIII	Effect of EEHI on epithelialisation in	
	different days	44
IX	Wound areas of different groups in excision	
	wound model	45

ACKNOWLEDGEMENT

INTRODUCTION

REVIEW OF LITERATURE

MATERIALS AND METHODS

RESULTS

DISCUSSION

SUMMARY AND CONCLUSION

REFERENCES

APPENDICES

CONTENTS

INTRODUCTION

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

Researchers have great interest in those substances which are derived from plants because they are versatile in their applications. Various phytochemicals can be obtained from plants which are very beneficial for mankind and medicinal plants have become the richest biological resource of such chemicals which are used in manufacturing of traditional drugs as well as in modern nutraceuticals, food supplements, medicines, folk medicines, raw material and pharmaceutical intermediates for synthetic drugs (Tumwine, 2011). Wound healing disorders present a serious clinical problem and are likely to increase since they are associated with diabetes, hypertension and obesity. Thus, several animal models have been established to serve as experimental basis to determine the mechanisms underlying and controlling undisturbed healing process (Kämpfer, 2003).

Wound healing is a multifactorial physiological process of repairing injured tissues that involves several cell types that result into restoration of a physical barrier (Nayak *et al.*, 2007). Impaired wound healing due to infection or metabolic complications can result into severe morbidity leading to long hospitalization of patients. The aim of treating wounds is to shorten the time taken for healing and to reduce risks of undesired complications. Plants have been found to synthesize compounds that are useful in the process of wound healing (Kalyon *et al.*, 2009).

The use of plants and plant products in the management of diseases has particularly been fueled by the rising cost of synthetic drugs. Plants have immense potential in management of wounds especially for people living in resource limited nations (Raina *et al.*, 2008). In Uganda, *H.indicum* L. (*Boraginaceae*) is used in traditional medicine for treatment of wounds; however there is no data to enable their development into clinically useful products. The present study was undertaken to determine the wound healing potential of the ethanol extracts of *H.indicum* L. in Wistar albino rats to generate preclinical data a step towards development of formulations and trial in clinical setting.

The simplest form of skin wound healing occurs when uninfected incisions and other clean wounds without loss of tissue are closed promptly by surgical sutures and this is referred to as healing by primary intention. When the healing of an open wound with loss of tissue occurs by the formation of more substantial amounts of granulation tissue which grows from the base of the wound to fill the defect, this is called healing by secondary intention (MacSween *et al.*, 1995). The first phase of wound healing is hemostasis in which the wound fills with the blood clot. The inflammatory phase follows with exudation of fluids, deposition of further fibrin and migration of neutrophil polymorphs, monocytes and lymphocytes. Then, the proliferative phase which is characterized by angiogenesis, collagen deposition, granulation tissue formation, wound contraction and lastly remodeling (Midwood, 2004).

Excised wound is aided by contraction of the surface area at sites where the skin is mobile and loosely attached to underlying tissue (Williams, 2004). Myofibroblasts, which are similar to smooth muscle cells, are responsible for contraction (Alam *et al.*, 2011). The basic mechanisms of healing by primary and secondary intention are similar. However, there is a greater connective tissue response in healing by secondary intention and the whole process is much slower. Treatment of infection accelerates wound healing because infection impairs the healing process by delaying which is important for granulation (Kämpfer, 2003). Herbal products have been used in treatment and healing of wounds over the years.

The phyto-medicines for wound healing are not only cheap and affordable but also purportedly safe since hyper sensitive reactions are rarely encountered with the use of these agents (Raina *et al.*, 2008). Thus, these products need to be identified and formulated for treatment and management of wounds.

Heliotropium indicum L. (Family: *Boraginaceae*) commonly known as 'Indian heliotrope' is very common in India with a long history of traditional medicinal uses in many countries in the world. An ethnopharma-cological survey revealed that, the traditional healers in Kancheepuram district of Tamil Nadu, India use *Heliotropium indicum* L. to cure skin diseases, poison bites, stomachache and nervous disorders (Chellaiah *et al.*, 2006).

Heliotropium indicum L. is believed to be useful in treating malaria, abdominal pain and dermatitis (Togola *et al.*, 2005). The decoction of the entire plant is taken orally for treatment of intractable fever, ulcers, venereal diseases and sore throat and used externally in vaginal cavity to induce abortion in pregnant females and administered rectally to treat local sore in the rectum and orally as diuretic and for the treatment of kidney stone (Berhault, 1974).

Villagers traditionally use the poultice prepared from different crude extracts to treat a variety of skin ailments including wounds. Wound healing process involves several steps, which involves coagulation, inflammation, formation of granulation tissue, matrix formation, remodeling of connective tissue, collagenization and acquisition of wound strength. During the formation of new tissue, endothelial cells proliferate and form new blood vessels.

There are several reports stating that the extracts of several plants, used for wound healing properties (Diwan *et al.*, 1982; Udupa *et al.*, 1989; Suguna *et al.*, 1996; Saha *et al.*, 1997; Sunilkumar *et al.*, 1998; Rasik *et al.*, 1999; Mukherjee and Suresh, 2000; Park and Chun, 2001; Nagappa and Cheriyan, 2001).

It was observed that *Aloevera* increased the collagen content of the granulation tissue as well as its degree of cross-linking as seen by increased Aldehydes content and decreased acid solubility (Kaufman *et al.*, 1988; Chithra *et al.*, 1998). *Aloevera*, *Channa striatus*, were also thought to have positive influence on the synthesis of glycosaminoglycan and thereby beneficially modulate wound healing (Chithra *et al.*, 1998b; Baie and Sheikh, 2000). By topical application of 0.2% solution of asiaticoside isolated from *Centella asiatica* produced 56% increase collagen content and better epithelisation (Shukla *et al.*, 1999), but *Curcuma longa* aqueous drops definitely delayed healing of superficial corneal wounds and delayed healing of penetrating corneal wounds also markedly reduced the tensile strength of corneal wounds (Mehra *et al.*, 1984). In the present study ethanol extracts of *Heliotropium indicum* L. were studied for wound healing properties in rats. Pharmacologically important plants present a large source of antimicrobial agents and serve as a drug in many countries (Mahesh & Satish, 2008). This antimicrobial activity of plant is due to their constituents of oils and extracts which are used in pharmaceutical and other related fields (Hammer *et al.*, 1999).

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

It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases and others (Young and Woodside, 2001). A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties.

Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems (Halliwell, 1994). The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is anti oxidative defense mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties.

The studies carried out on medicinal plants and vegetables strongly support the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems. In the present investigation, we studied ethanol leaf extracts for the search of wound healing activity of *Heliotropium indicum* L.

Plants depend upon radiant energy for the energy necessary to carry on photosynthesis and other physiological processes. The green plant has been called the converter of solar energy. In the presence of sunlight it synthesizes complex organic compounds such as sugars, fats, proteins, etc., from simple inorganic compounds such as water, carbon dioxide, minerals, salts, etc. The interaction of plants with radiant energy is of interest to the botanist, forester, geographer, biophysicist, biochemist, ecologist, hydrologist and agronomist (Gates *et al.*, 1965).

Therefore, the analysis of bioactive constituents in plants using spectral studies would help in determining various biological activities of plants. The determination of phytoconstituents is largely performed by relatively expensive and often laborious techniques such as gas and liquid chromatography combined with specific detection schemes (Uzer *et al.*, 2005 & Eisenhauer *et al.*, 2009).

In the present study, simple, cost-effective and rapid tests for detecting phyto components are used. Spectroscopic ultraviolet-visible, Fourier transform infrared (UV-Vis, FT-IR methods together or separate can be used in this sense as well as conventional methods (Hazra *et al.*, 2007 & Eberhardt *et al.*, 2007). UV-Vis spectrophotometer related to the spectroscopy of photons in the UV-visible region. UV-Vis spectroscopy uses light in the visible ranges or its adjacent ranges.

The color of the chemicals involved directly affects the absorption in the visible ranges. The FT-IR has proven to be a valuable tool for characterization and identification of compounds or functional groups (chemical bonds present in an unknown mixture of plants extract (Aysal *et al.*, 2007 & Ibrahim *et al.*, 2008). In addition, FT-IR spectra of pure compounds are usually so unique that they are like a molecular fingerprint.

The therapeutic effect of medicinal plants for the treatment of various diseases was based on the chemical compounds of these plants. The major components are organic compounds, some of which have biological activity, but none act independently and cannot replace the functions of the medicinal plant as a whole. Analysis has revealed that medicinal plants are rich in many trace elements, and it is suggested that this is an important factor in the curative effect of these plants (Olabanji *et al.*, 1997; Singh *et al.*, 2012; Pereira and Felcman, 1998).

The chemical states in which trace elements found are originally bound, completed and free; besides, different states have different functions, toxicity, and absorption rates by the body. Trace elements co-exist with numerous organic compounds many of which are complex agents in the infusions of medicinal plants (Remington, 1995) and most will be bound to organic compounds, therefore, the concentration of the free trace elements can be very low. The continuing interest in trace elements has stimulated the development of several powerful analytical techniques for their detection and quantitative 7

determination. One of these is X-ray diffraction XRD, where a source of X-ray photons is used to study the elemental composition of materials. Absorption of these photons by the photoelectric effect produces vacancies in the inner electron shells of the atoms of the material, followed by the emission of characteristic X-rays of the elements present. The peaks in X-ray spectrum indicate what kinds of atoms are present, while the number of counts the area under the peak in relates to the number of atoms in the sample, alluring the quantitative measurements to be made (Burham, 1984).

Moreover, the method gives information about the elements present in the sample irrespective of their state of chemical combination or the phases in which they exist (Piorek, 1978). The chemical constituents of most of the plants used by traditional healers are still unknown. It is, therefore, of paramount importance to know the chemical composition of every reported plant and to make permanent records of the knowledge from natural sources before they all pass away. Hence in the present study, an attempt was made to create a permanent record of the chemical constituents of the leaves of *Heliotropium indicum* L. by evaluating the antimicrobial, wound healing activity and phytochemical analysis.

With this background, we have formulated the following objectives

1. To study the wound healing effect of *Heliotropium indicum* L. leaves using *in vivo* model.
2. To analyze the major phytochemical components present in the leaves extract of *Heliotropium indicum* L.
3. To evaluate the antimicrobial activity of leaves extract of *Heliotropium indicum* L. in different solvent systems.

2. REVIEW OF LITERATURE

The review of literature pertaining to the study “**Evaluation of wound healing activity of *Heliotropium indicum L.* leaves**” is presented in the following headings:

2.1. Natural plant product research

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

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

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

and with herbs from Chinese and Indian traditional medicines (Cragg and Newman, 2013).

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

During this period, medicinal plants were only applied on an empirical basis, without mechanistic knowledge on their pharmacological activities or active constituents. Rational drug discovery from plants started at the beginning of the 19th century, when the German apothecary assistant Friedrich Serturmer succeeded in isolating the analgesic and sleep inducing agent from opium. This trend resulted in the examination of other medicinal herbs, and during the following decades of the 19th century, many bioactive natural products, primarily alkaloids (e.g., quinine, caffeine, nicotine, codeine, atropine, colchicines, cocaine and capsaicin) could be isolated from their natural sources (Corson and Crews, 2007; Felner and Lloyd, 1898; Hosztafi, 1997; Kaiser, 2008; Kruse, 2007; Sneader, 2005).

These efforts were then followed to produce natural products by chemical synthesis aimed at production at higher quality and lower costs. Salicylic acid was the first natural compound produced by chemical synthesis in 1853 (Kaiser, 2008). After the discovery of penicillin (1928), an era of drug discovery from microbial sources was initiated in the 1930's. This paved way to the scientific and financial foundation of the modern pharmaceutical industry after World War II. Despite the advent of combinatorial chemistry and High Throughput Screening (HTS) campaigns during the last decades, the impact of natural products for drug discovery is still very high. Of the 1073 new chemical entities belonging to the group of small molecules that had been approved

between 1981 and 2010, only 36% were purely synthetic, while more than the half were derived or inspired from nature (Newman and Cragg, 2012).

A substantial number of these compounds have been discovered in higher plants, anti-cancer agents, e.g., paclitaxel and its derivatives from yew (*Taxus*) species, vincristine and vinblastine from Madagascar periwinkle (*Catharanthus roseus* (L. G. Don), and camptothecin and its analogs initially discovered in the Chinese tree *Camptotheca acuminata* Decne (Cragg and Newman, 2013; Kinghorn *et al.*, 2011) and the important anti malarial and potential anti cancer agent artemisinin originally derived from the traditional Chinese herb *Artemisia annua* L.

2.2. Traditional medicine

Traditional medicine (TM) holds great potential to improve people's health and wellness. It is an important, yet often underestimated part of health care. TM is found in almost every country in the world and the demand for its services is increasing every day.

TM can contribute to addressing a number of global health challenges of the 21st century, in particular in the area of chronic, non communicable diseases and population aging. Traditional medical knowledge is experiencing increased attention worldwide in light of global health care demand and the significant role of traditional medicine in meeting the public health needs of developing countries.

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

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

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

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

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

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

the introduction of microbiological science and the separation of medicine from pharmacological science.

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

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

2.3. Phytochemicals and its significance

Phytochemicals are often regarded as “alternative” therapies widely used in mainstream medicine and compose the primary therapeutic strategies for numerous conditions (including cancer). A significant proportion of the current pharmaceutical market is derived from naturally occurring plant compounds. In the last decade alone, more than 20 newly approved drugs were derived from natural sources, including plants and microorganisms (Sarkar, 2010). It has been estimated that 50% of contemporary are either

directly extracted from plants or chemically derived from naturally occurring compounds (Amin *et al.*, 2009). Notable examples include the opioid analgesics, which are derived from the latex sap of the opium poppy (*Papaver somniferum*) and compose one of the best selling classes of prescription medications (Brownstein, 1993). Another example is aspirin (acetylsalicylic acid), the world's first- ever synthetic drug, derived from salicylic acid, a constituent found in willow (*Salix spp.*) and meadowsweet (*Filipendula ulmaria*). It has been called "the most popular pain killer in the world" and has shown efficacy in the prevention of colon cancer (Jack, 1997)

Flavonoids are the most diverse group of phytochemicals. Research suggests that flavonoids, in particular, may be an important phytochemical group that contributes to the reduced mortality rates observed in people consuming high levels of plant-based foods. In the Zutphen elderly study, findings revealed a significant inverse association between flavonoids intake and myocardial infarction. Similarly, findings from the seven countries study (comparing the diets of men living in Finland, Italy, Greece, the former Yugoslavia, Japan, Serbia, the Netherlands, and the United States) suggested that consumption of flavonoids was responsible for 25 percent of the observed difference in mortality rates in various countries (Hertog *et al.*, 1995).

Flavonoids are widely occurring polyphenolic compounds and are extremely important because of their medicinal effects. Flavonoids from different species of Solanun have been reported and reviewed (DaSilva *et al.*, 2003). Also they have been isolated and characterized from fruits such as grapes and apples, vegetables such as broccoli and onion, spices such as turmeric, beverages such as green tea and red wine, as well as many other sources (Bohlin and Bruhn, 1999).

Flavonoids and tannins as phenolic compounds and other plant phenolics are major group of compounds that act as primary antioxidants or free radical scavengers (Saxena and Saxena, 2012). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al.*, 2007). They possess biological properties such as anti-apoptosis, anti-

aging, ant cancer, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al.*, 2007).

The plant phenols are regarded as those substances derived from the shikimate pathway and phenyl propanoid metabolism, following the phosphoenol pyruvate, phenylalanine, cinnamate, 4-coumarate, leading to chalcone, flavanone, dihydroflavonol and anthocyanin (Roberts and Antolovich, 1997). Many of these phenolic compounds are essential to plant life, e.g., by providing defence against microbial attacks and by making food unpalatable to herbivorous predators (Bennick, 2002). Previous studies have found that phenolic compounds are major antioxidant constituents in selected herbs, vegetables and fruits, and there are direct relationship between their antioxidant activity and total phenolic content (Dorman *et al.*, 2004).

Alkaloids are organic compounds containing nitrogen, which have obvious biological activity and are mostly present in plants. Alkaloids have a wide distribution in the plant kingdom and mainly exist in higher plants, such as those belonging to *Leguminosae*, *Papaveraceae*, *Loganiaceae* and *Menispermaceae*. Plant-derived alkaloids currently in clinical use include analgesics, anti- neoplastic agent, gout suppressant, muscle relaxants, antiviral, cytotoxic, anticholinergic, anti-inflammatory and DNA-binding activities and some of them have also been used in the treatment of Alzheimer's disease, myasthenia gravis and myopathy (Crozier *et al.*, 2006). Variety of saponins with complex structure widely exists in plants.

The most widely studied is the ginsenoside. Ginsenoside is a kind of triterpenoid, saponins, and it is the main active ingredients in ginseng. A large number of studies have shown that ginsenoside has higher anti- tumour activity, non- toxic side effects on normal cells and has a synergistic effect with other chemotherapy drugs such as cisplatin. Ginsenoside regulates the proliferation of tumor cells, inducing differentiation and apoptosis of cells to exert antitumor effects. Lignans are phenolic plant compounds found in high

concentrations in flax and sesame seeds and in lower concentrations in grains, other seeds, fruits and vegetables.

Lignans are stereo specific dimers of cinnamic alcohols (monolignols) bonded at carbon 8 (C8-C8) that are derived in the phenylpropanoid pathway and formed through phenolic oxidative coupling processes. Lignans (monolignol dimmers) usually occur in the free state or bound to sugars, and diglucosides of pinoresinol, secoisolariciresinol, and syringaresinol are common. Lignans, neolignans and their analogues are involved in plant defence (as antioxidants, biocides, phytoalexins, *etc.*), provide protection against diseases and pests and possibly participate in plant growth control (Peterson *et al.*, 2010).

2.4. *Heliotropium indicum* L. – an overview

Heliotropium indicum L. (Family: Boraginaceae) commonly known as 'Indian heliotrope' is very common in India with a long history of traditional medicinal uses in many countries in the world. An ethnopharma-cological survey revealed that, the traditional healers in Kancheepuram district of Tamil Nadu, India use *Heliotropium indicum* L. to cure skin diseases, poison bites, stomachache and nervous disorders (Chellaiah *et al.*, 2006).

Heliotropium indicum L. is believed to be useful in treating malaria, abdominal pain and dermatitis (Togola *et al.*, 2005). The decoction of the entire plant is taken orally for treatment of intractable fever, ulcers, venereal diseases and sore throat and used externally in vaginal cavity to induce abortion in pregnant females and administered rectally to treat local sore in the rectum and orally as diuretic and for the treatment of kidney stone (Berhault, 1974).

It is a coarse foetid herb, up to 2 ft. high, with ascending hirsuit branches found throughout India in sunny localities, on waste lands and anthropogenic habitats in periodically desiccating pools and ditches and anthropogenic habitats, generally below 800 m altitude, widely considered as a weed of fields. The leaves are simple, alternate or sub-opposite, 4.5 to 10 cm/2.5 to 5 cm,

ovate or ovate oblong, and margin undulate, sparsely strigose along nerves on either side, serulate or undulate with cordate, minutely pilose beneath nerves and veins conspicuous on the lower side.

The active principle responsible for the antitumor activity is reported to be indicine N-oxide (Srinivas, 2000). The aqueous extract of the leaves is reported to possess strong uterine stimulant effect (Barros *et al.*, 1970). Earlier reports on wound healing activity of the plant have been reported on the basis of only preliminary animal models like excision and incision wound models. The present study was undertaken to provide an in depth study on wound healing activity of the leaves using all possible models and to provide a scientific support to its use in the folklore medicines for treating wound infection.

H. indicum L. may flower throughout the year; the flowering season is very long and new flowers develop apically within the cyme while mature nutlets are already present at the base of the inflorescence. The flowers are white or violet coloured, regular, sessile, two ranked pentamerous, extra axillary. Numerous branched, more or less densely hirsute with spreading hairs are found in the stem and the root system is tap root and branched (Kirtikar *et al.*, 1994)

2.4.1. Ethno pharmacology

H. indicum L. has been used in different traditional and folklore systems of medicine for curing various diseases. An ethnopharmacological survey revealed that, the traditional healers in Kancheepuram district of Tamil Nadu, India use *H. indicum* L. to cure skin diseases, poison bites, stomachache and nervous disorders 6. In some African countries, another ethnopharmacological survey reports that *H. indicum* L. is believed to be useful in treating malaria, abdominal pain and dermatitis. The highest number of usages 22% was reported for the treatment of malaria (Togola *et al.*, 2005)

In Jamaica, the decoction of the entire plant is taken orally for treatment of intractable fever, ulcers, venereal diseases and sore throat and used externally in vaginal cavity to induce abortion in pregnant females and administered rectally to treat local sore in the rectum (Asprey and Thornton *et al.*, 1995) while in Philippines and Senegal, used orally as diuretic and for the treatment of kidney stone (Berhault , 1974).

The infusion of the flower is taken orally by females for the treatment of menorrhagia in Jamaica. In Rodrigues, the decoction of the entire plant is used externally for treating herpes and the paste of fresh plant is used externally for cleansing and dressing of wounds and ulcers. The sap of the stem is used orally by females for treating dysmenorrhea (Fakim *et al.*, 2000) The hot water extract of the flower is taken orally by the females as an emmenagogue in small dose and abortive in large dose while a paste of fresh entire plant is used externally for treatment of head lice in the West Indies (Ayensu ,1978)

In Thailand, the dried inflorescence is believed to produce permanent sterilization when taken orally in females. One gram of the dried and powdered inflorescence mixed with milk or water is used for three days beginning with the fourth day of menses to achieve the desired result (Panthong *et al.*, 1986) Other folk remedies include use of decoction of the leaves for treatment of fever, insect bites, stings, diarrhoea, skin rashes, menstrual disorder and urticaria (Dutttagupta and Dutta, 1977).

The decoction of the leaves is also credited to be useful in curing insect stings (macerated with sugar cane juice, scorpion stings 10 and as abortive in large dose and emmenagogue in small dose (Barrett, 1994). The leaf paste is applied externally to cure rheumatism in Rayal Seema in Andhra Pradesh, India and skin infection in Nicaragua.

The decoction of both leaf and root together is also used for treating whooping cough in children in Eastern Nicaragua (Coe and Anderson, 1996). In Amazon, the paste of both leaf and root together is applied externally in scorpion stings, bug bites while the paste is recommended for treating sores and warts in Taiwan (Lin and Kan, 1990).

In Malaysia, a paste made from the plant is applied to counteract putrefaction, to treat pyoderma and ringworm infection. In Burma, a decoction of the whole plant is used to treat gonorrhoea while in Indonesia; an infusion of the leaves is used to soothe mouth sprue. A decoction of the dried roots is drunk in the Philippines to promote menses, while the seeds are used to treat cholera, malaria, and for wound-healing (Wiar, 2006).

2.4.2. Scientific classification of *Heliotropium indicum* L.

Kingdom	:	<u>Plantae</u>
Clade	:	<u>Angiosperms</u>
Clade	:	<u>Eudicots</u>
Clade	:	<u>Asterids</u>
Order	:	<u>Boraginales</u>
Family	:	<u>Boraginaceae</u>
Genus	:	<i>Heliotropium</i>
Species	:	<i>indicum</i>

2.4.3. Vernacular names of *Heliotropium indicum* L.

Common name of *Heliotropium indicum* L. is Indian Heliotrope, In Indian turnsole. In Bengali it is called as Hatisura. In Gujarati, Hathi-sundhara, Hindi Hathajori, Kannada: Chelubalada Gida. In Konkani - Ajeru, Malayalam: Thekkada, Thekka, Therkkada, Thekkida. Manipuri people called as Leihenbi, Marathi: Bhurundi, Oriya: Hati-sand, In Sanskrit it is called as Vrscikali, Hastishundi, Shrihastini, Chanchuphala, In Tamil: Tetkotukki, Telugu: Naga Danti, Nepali: Haattisunde Jhaar.

2.5. Therapeutic value – *Heliotropium indicum* L.

Traditional healers in Kancheepuram district of Tamil Nadu, India topically apply paste of whole plant to treat wounds and skin infections (Muthu *et al.*, 2016). In Kumaragiri Hills of Salem district, Tamil Nadu, India, leaf juice mixed with hot water is applied to snake bite and scorpion sting (Alages aboopathi, 2009). Multiple uses of the plant have been reported from Africa. In Mali, the plant is used for treatment of vomiting, amenorrhoea, baby thinness,

ocular infections, and high blood pressure. Leaf decoction is used in Sierra Leone for washing new-borne babies. Sap is applied to gumboils, to clean ulcers, and to cure eye infections in Nigeria and Ghana.

Decoction of whole plant is taken as febrifuge in Guinea. Leaf powder is used in Senegal for dermatitis, suppurating eczema and impetigo in children. In Ivory Coast, dry leaf powder is taken nasally as decongestant for colds and sinusitis. Besides these African countries, in Indonesia, leaf decoction is used in thrush and in poultices for herpes and rheumatism (Togola *et al.*, 2005). Other uses of the plant as reviewed by Dash and Abdullah (2013), include using orally decoction of entire plant in Philippines and Senegal as diuretic and for treatment of kidney stone (Quisumbing, 1951; Berhault, 1974)

Infusion of flower taken orally in Jamaica for treatment of menorrhagia (Asprey and Thornton, 1955); decoction of the entire plant is also taken orally in Jamaica for intractable fever, ulcers, venereal diseases, sore throat, and applied to vaginal cavity to induce abortion and administered rectally to treat rectal sores; decoction of entire plant used in Rodrigues for treating herpes and using paste of fresh plant for cleansing and dressing of wounds and ulcers. In West Indies, paste of fresh entire plant is used for head lice (Ayensu, 1978).

In Thailand, dried inflorescence oral administration is believed to produce permanent sterilization in females (Panthong *et al.*, 1986). Leaf paste is applied topically for skin infections in Nicaragua; decoction of a combination of leaf and root is used for treatment of whooping cough in children in Eastern Nicaragua (Barrett, 1994; Coe and Anderson, 1996). The plant is also used in South America, where in the Amazon region, paste of leaf and root together is applied externally to insect bites and scorpion stings (Duke, 1994). However, the same paste is applied for treating hepatitis in Taiwan (Lin and Kan, 1990).

Leaves and stems of *Heliotropium indicum* L. are used as antidote to poisoning and leaf paste to treat bone fractures by folk medicinal practitioners in Rajshahi district, Bangladesh (Nawaz *et al.*, 2009). In Patna district of Bangladesh, a folk medicinal practitioner has been reported to use the roots of

this plant to treat swelling of knees, joint pain, and severe itching in leg joints accompanied by oozing of fluid from the joints (Kamal *et al.*, 2014).

Folk medicinal practitioners in Manikganj district, Bangladesh use root juice of the plant to treat chicken pox and leaf juice to treat allergy (Shahnaj *et al.*, 2015). In Natore district, Bangladesh, a folk medicinal practitioner uses the root for blood purification and to treat infections (Akhter *et al.*, 2016).

Random surveys in various areas of Rajshahi and Tangail districts, Bangladesh were conducted among folk medicinal practitioners FMPs to find out other uses of *Heliotropium indicum* L. (Muthu *et al.*, 2016). In Rajshahi district, one FMP Idrish Ali, male, age 65 years used paste of leaves topically to stop bleeding from external cuts and wounds and to stop any pain occurring from bleeding (Akhter *et al.*, 2016). Amjad Hossain male, age 75 years applied leaf juice to eyes to clear blurred vision; Afroza Begum female, age 41 years used leaf juice to stop bleeding as well as for blurred vision; Md. Shahjahan Ali male, age 67 years used roots oral administration to stop abnormal bleeding during menstruation. (Panthong *et al.*, 1986).

The informants were all from Bagha Thana Police Station. In Tangail district, Toripon Nessa of Nagarpur Thana female, age 75 years mix leaf juice with a pinch of table salt and apply it to eyes once daily for 3-4 days as treatment for cataract, intraocular pressure and eye pain. Since *Heliotropium indicum* L. is a common plant in Bangladesh, specimen plants from both Rajshahi and Tangail were simply shown at the Bangladesh National Herbarium and made certain as to their being actually *Heliotropium indicum* L. Preliminary experimental studies suggest that aqueous whole plant extract may be beneficial in selenite induced cataract in rat pups (Kyei *et al.*, 2015) and galactose-induced cataract in Sprague-Dawley rats (Kyei *et al.*, 2017).

Cataract needs surgery, which can be expensive and also lead to post-surgical complications. Untreated high intraocular pressure (IOP) can lead to glaucoma and loss of eye-sight. Cataract remains the major cause of avoidable blindness in Bangladesh according to Bangladesh Government statistics. Glaucoma is another leading factor for blindness in Bangladesh. As

such, *Heliotropium indicum* L. can provide a novel and affordable solution for treatment of cataract and glaucoma.

2.6. Major chemical constituents and nutrients of *Heliotropium*

indicum L.

H. indicum L. is very rich in pyrrolizidine alkaloids. Numerous pyrrolizidine alkaloids have been identified in this plant by several authors. The alkaloids reported in the entire plant include heliotrine (Pandey *et al.*, 1996), lasiocarpine, indicine, 12-acetyl indicine, indicinine, indicine-N-oxide, retronecine, trachelan-thamide with traces of supinidine and lindelofidine. The aerial parts contain echinatine, heleurine, lasiocarpine-N-oxide, supinine, heliotrine, indicine, indicine-N-oxide and lasiocarpine (Mattocks, 1967)

Presence of cynoglossine, europine-N-oxide, heleurine-N-Oxide, heliotridine-N-Oxide, heleotrine-N-Oxide 29 and heliotrine 30 has been identified from the seeds. Other alkaloids such as putrescine, spermidine, homo spermidine and spermine have been identified in the leaves 31. Apart from alkaloids, several triterpenes and steroids including (Birecka *et al.*, 1984) -amyrin, lupeol, chalinasterol (Andhiwal *et al.*, 1985), -sitosterol, stigmasterol and campesterol have been reported from the entire plant. Other compounds reported from the entire plant include rapanone and hexacosan-1-ol. Presence of estradiol (Mannan and Ahmad, 1978) has been reported in the roots.

Helindicine, a new pyrrolizidine alkaloid together with the known lycopsamine were isolated from the roots of *H. indicum* L. The structures were established by a combination of 1D and 2D NMR methods COSY, HMQC, HMBC, and NOESY and HREIMS. This is the first report of a lactones pyrrolizidine alkaloid in the genus *Heliotropium indicum* L. Helindicine and lycopsamine were assayed for antioxidant activity and showed moderate activity (Souza, 2005).

The essential oil of *H. indicum* L. extracted by hydro distillation was analyzed by Gas chromatography GC and gas chromatography–mass spectrometry (GC-MS). Aldehydes (52.8%) occurred in the highest amount represented by phenyl acetaldehyde (22.2%), (E-2) nonenal (8.3%) and

(E, Z-2-)nonadienal (6.1%), with a significant quantity of hexahydrofarnesylacetone (8.4% 36). In another experiment, the volatile oil isolated by hydro distillation was analyzed by a combination of GC–FID and GC–MS. The major constituents of the volatile oil were phytol (49.1%), 1-dodecanol (6.4%) and β -linalool (3.0%) (Machan *et al.*, 2006).

The major elements, comprising calcium, phosphorus, sodium, potassium, magnesium and trace elements iron and zinc were determined according to the method of Shahidi *et al.* (1999). The ground plant samples were sieved with a 2 mm rubber sieve and 2 g of each of the plant samples were weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO₃/HCl/H₂O (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colorless solution was obtained.

The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through a whatman No. 42 filter paper and the volume was made to the mark with deionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer. A 10 cm-long cell was used and concentration of each element in the sample was calculated on percentage of dry matter.

Phosphorus content of the digest was determined colorimetrically according to the method described by Nahapetian and Bassiri (1975). To 0.5 ml of the diluted digest, 4 ml of demineralised water, 3 ml of 0.75M H₂SO₄, 0.4 ml of 10% (NH₄6MO7O₂₄.4H₂O) and 0.4 ml of 2% w/v ascorbic acid were added and mixed). The solution was allowed to stand for 20 min and absorbance readings were recorded at 660 nm. The content of phosphorus in the extract was determined.

Aliphatic alcohols and acids, amino acids and alkaloids, carbohydrates, carotenoids, hydrocarbons, free fatty acids including polyunsaturated fatty acids (PUFAs, lipids, pheromones, phorbol esters, Phenolic, and related compounds, steroids, triterpenes, and their glycosides, tannins, other terpenes and re-lated compounds, are among these classes). Among the latest additions are an array of substances from 423 gums and glues to alkaloids and Saponins and other substances of interest to modern industry and medicine. Chemicals such as amino acids, carbohydrates and proteins, are products of primary metabolism and are vital for the maintenance of life processes, while others like alkaloids, phenolics, steroids, terpenoids, are products of secondary metabolism and have toxicological, pharmacological and ecological importance (Bandaranayake, 2000).

2.7. Wound healing activity of *H.indicum* L.

Heliotropium indicum L. (Family: Boraginaceae) is a coarse foetid herb, up to 2 ft. high, found throughout India in sunny localities, on waste lands, and anthropogenic habitats, widely considered as a weed of fields (Chadha *et al.*, 1985). The plant is reported to be highly valued in the folklore medicine and is believed to be useful in treating malaria, abdominal pain, fever, dermatitis, venereal diseases, insect bites, menstrual disorder, urticaria, and sore throat (Muthu *et al.*, 2006).

The plant decoction is considered as diuretic and remedy for the treatment of kidney stone (Quisumbing *et al.*, 1951). The leaf paste is applied externally to cure rheumatism and skin infections (Nagaraju *et al.*, 1990). The various tribes of Phulbani district of Odisha use the leaf paste over fresh cuts and wounds and claim for its promising activity.

Earlier reports on wound healing activity of the plant have been reported on the basis of only preliminary animal models like excision and incision wound models. The present study was undertaken to provide an in depth study on wound healing activity of the leaves using all possible models and to provide a scientific support to its use in the folklore medicines for treating wound infection.

Infections are known as one of the most important factors influencing efficiency of wound healing. It is reported that a high percentage of wound related complications and hence costs in wound care, can be directly linked to infected wounds (Care, 2004). Reducing the bacterial load may be one of the most important necessary requirements for better wound healing, since wound infection either secondary or primary by opportunistic microorganisms can lead to reduction of local inflammation and consequentially avoid tissue destruction (Young and McNaught, 2011). An ideal medicine for prevention of wound infection should act antimicrobial and at the same time stimulate the body's natural immune activity without damage to surrounding healthy tissue (Gantwerker and Hom, 2012).

Most wounds induce sensation of pain. Pain relates to patient discomfort, release of stress factors and often reduces the patients' quality of life. Hindered mobility, psychological issues connected with pain induced stress and of course the financial burden, caused by prolonged therapy, all contribute to less effective wound healing. According to (McGuire *et al.*, 2003) chronic pain lowers the patients' capability of healing, prolonging the overall recovery process (*PlastSurg* 2012). Suitable and effective pain management can lead to an earlier ambulation, adequate oxygenation, nutrition and stress reduction.

All mentioned results in facilitation of wound healing, while minimizing risk for development of chronic pain, as well as finally in lowered treatment costs (Midwood *et al.*, 2004). Inflammation causes also a coordinated influx of neutrophil to the wound site. Neutrophil activate their so called "respiratory burst" and produce free radicals (Kirsner *et al.*, 1993).

The presence of free radicals results in oxidative stress leading to lipid peroxidation, DNA breakage, and enzyme inactivation, including free-radical scavenger enzymes, which are known to limit the effects of reactive oxygen species ROS. Evidence for the role of oxidants in the pathogenesis of many diseases suggests that antioxidants may be of therapeutic use in these conditions; hence improve efficiency of wound healing (Myers *et al.*, 1980).

III MATERIALS AND METHODS

The materials used and methods adopted in the present study entitled “**Evaluation of Wound Healing Activity of *Heliotropium indicum* L. leaves**” is presented in the following headings

3.1. Plant material and extract preparation

The leaves of *Heliotropium indicum* L. were collected from Sirumugai, Coimbatore. The leaves were shade dried and powdered using an electric blender. The fine powder 20g was suspended in 200ml of petroleum ether, ethanol, chloroform, ethanol, acetone for 20 hours at room temperature. The mixture was filtered by using whatman’s filter paper. The filtrate was placed in a water bath to dry at 40°C and the obtaining clear residue was used for the study. The extracts were used for preliminary phytochemical screening of the different extracts to know the phytochemical constituents.



Fig.1. *Heliotropium indicum* L. plant



Fig.2. *Heliotropium indicum* L. leaves powder

3.2. Experimental animals

Animal wound healing models are important biological tools to understand basic processes of tissue repair and to develop and validate strategies for clinical treatment. Human wound healing has many unique aspects that relate to the physiology, age and environment of the species, but the opportunity to carry out controlled, clinical experimentation on the mechanism and therapy of wounds is limited. In general, animal models with the exception of some transgenic and targeted gene deletions attempt to reflect human wound healing problems, dehiscence, ischemia, ulceration, infection, and scarring.



Fig.3. Experimental animals (Wistar albino rats)

Healthy wistar albino rats (150 to 200 gm) were used for the study. The animals were obtained from Avinashilingam Institute for Home Science and Higher Education for Woman, Coimbatore and brought to the laboratory. Animals were kept in polypropylene cages with sawdust bedding and maintained in laboratory conditions. Standard pellets were given as diet and water was provided to the animals. The animals were acclimatized to laboratory condition for about one week before commencement of the experiment. The experiments were performed after the approval from the institutional Animal Ethical Committee (IAEC No: KMCRET / M.Sc. / 2018 - 2019 and in accordance with the recommendation for the proper care and use of the laboratory animals.

3.2.1. Chemicals required

Simple ointment, Ketamine anaesthesia, ethanol, paraffin, petroleum ether, chloroform and acetone

3.2.2. Drug formulation

0.5 g of EEHI was mixed with 5.5 gm of Paraffin wax and transferred to a tightly closed container. Two types of drug formulations were prepared for topical administration 5% w/w ointment was prepared in simple ointment base. For dorsal administration, 200mg/kg suspensions of the extract were prepared. The drugs were applied and wound healed area. For assessment of wound healing activity, excision model were used. The animals were divided into four groups of 4 animals each used for excision wound model respectively.

3.2.3. Wound healing activity

Excision wound model was used to evaluate the wound healing activity of *Heliotropium indicum* L. leaves.

3.2.4. Excision wound model

Animals were divided in to four groups (Fig.5) each comprising four animals. Four groups were injected with ketamine anastasia to create wound. The treatments were given dorsal fur of the body at 24 h after the wound healing activity continued for 21 consecutive days.

In the Excision wound model the animals were grouped as follows:

- Group I** : Served as control for 21 days.
- Group II** : T – Bact (Simple Ointment) I.P. twice a day for excision model for 21 days.
- Group III** : 200 mg/kg of the EEHI extract mixed with paraffin wax base twice a day for excision model for 21 days.
- Group IV** : 200 mg/kg of the EEHI extract twice a day for excision model for 21 days.

EEHI : Ethanol extract of *Heliotropium indicum* L.

All the treatments were continued for 21 days. The wound closure of all animals in all groups was measured respective days. After the final closure rate, five animals of each group were sacrificed to study the wound closure rate of the animal.

3.2.5. Epithelialisation period

It was monitored by observing the number of days required for escher to fall away, leaving no raw wound behind.

3.2.6. Wound contraction

To monitor this, progressive changes in wound area were followed planimetrically. Leaving the wounding day, wounds were measured on alternate days.

The animal was restrained in proper position during measuring. From this, wound areas were read and the percent of wound contraction was

calculated taking the initial size of wound (100 mm² as 100%). Percentage wound closure can be calculated using the formula as follows

$$\text{Percentage wound contraction} = \frac{\text{Healed Area}}{\text{Total Area}} \times 100$$

3.3. Statistical analysis

The mean value \pm SEM was calculated for each parameter. Results were statistically analyzed by one way analysis of variance (ANOVA) followed by DMRT. $P < 0.05$ was considered as significant.

3.4. Antimicrobial activity

3.4.1. Test organisms

Staphylococcus aureus, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Rhizopus* and *Aspergillus niger* was tested. The sensitivity of different strains of extracts was measured zone of inhibition by using an agar diffusion assay. Organisms with a clear zone of inhibition of more than 12mm were considered to be sensitive.

3.4.2. Methodology

Antibacterial and antifungal activity studies were carried out by agar diffusion method (Barry *et al.*, 1976). Standard Ampicilin (50 μ g of Nutrient agar) was used as the standard reference drug for antibacterial assay, Fluconazole (50 μ g) was used for antifungal activity study (sarkar *et al.*,1998).The pure cultures of different pathogens were grown overnight in sterile nutrient broth and incubated at 37°C for 24 hours.

The 0.1ml of the culture was seeded on 25 ml of solidified nutrient agar and rose bengal plates for bacterial and fungal cultures, respectively. The wells were bored with 8mm borer in seeded agar, and then the particular concentrations (50 μ l) of the extracts were added in each well. Soon after the plates were then kept at 10°C for 30min. After it normalized to room

temperature plates were incubated at 37°C for 24hrs. After incubation period is completed, the zone of inhibition was measured and recorded.

3.4.3. Statistical analysis

The values of antimicrobial activity of the different leaves extract of *H. indicum* L were expressed as mean \pm standard deviation (n= 4) for each sample.

3.5. Phytochemical screening

Preliminary screening of the extracts and identification was done by color tests adapting standard methods by Raman (2006).

3.5.1. Test for alkaloids

Mayer's test

A fraction of extract was treated with Mayer's test reagent 1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml of water and observed for the formation of cream colour precipitate.

Wagner's test

A fraction of extract was treated with Wagner's reagent 1.27 g of iodine and 2 g of potassium iodide in 100 ml water and observed for the formation of reddish brown colour precipitate.

Dragendroff's test

1ml of the extract was added to 1ml of Dragendroff's reagent. Appearance of orange colour precipitation indicates the presence of alkaloids.

3.5.2. Test for flavonoids

Alkaline reagent test

1 ml of the extract was treated with aqueous NaOH and HCl. The formation of yellow orange colour indicates the presence of flavonoids.

Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

3.5.3. Test for sterols

Liebermann- burchard test

Extract 1ml was treated with chloroform, acetic anhydride and drops of H₂SO₄ was added and observed for the formation of dark pink or red colour.

Salkowski

When concentrated sulphuric acid was added to a chloroform solution to the extracts 10 mg of extract in 1 ml of chloroform, a reddish blue colour was produced in the chloroform layer and green fluorescence in acid layer, suggesting the presence of steroids.

3.5.4. Test for phenols

Ferric chloride test

The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour

3.5.5. Test for saponins

Foam test

To the extract, 20ml of distilled water was added and agitated on a graduated cylinder for 15 minutes. The formation of about 1 cm layer of foam indicates the presence of Saponins

3.5.6. Test for tannins

Gelatin test

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

3.5.7. Test for proteins

Ninhydrin test

A small quantity of extract solution was boiled with 0.2% solution of Ninhydrin. Blue colour indicates the presence of amino acids.

Molisch's Test

To small quantities of solvent free ethanol extract, few drops of 1% α -naphthol in ethanol were added. Conc. H_2SO_4 was then added to sides of the 24 test tubes. A brown purple ring formed at the junction of the two liquids indicates the presence of sugars.

Fehling's test

Small quantities of solvent free ethanol extract were separately dissolved in minimum amount of distilled water and filtered. To the filtrates equal volume of Fehling's solution were mixed in a test tube separately and heated for few minutes. Formation of brick red precipitate confirmed the presence of sugars.

IV RESULTS

The results pertaining to the study “**Evaluation of Wound Healing Activity of *Heliotropium indicum* L. leaves**” are presented in the following headings

4.1. Preliminary phytochemical screening of *Heliotropium*

***indicum* L. leaves**

The phytochemical constituents serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Investigations on secondary plant constituents have made phenomenal advance during the past few decades. Based on the above concept few analysis were done with the extracts used in the present study which are described below (Table I).

Table I. Preliminary phytochemical analysis of *Heliotropium indicum*

L. leaves

S.NO	Constituents	Test for Constituents	Solvents				
			ET	CH	PE	AQ	AC
1.	Alkaloids	1.Mayer's Test	+	-	-	+	-
		2.Dragendroff's Test	+	+	+	-	-
		3.Wagner's Test	+	-	-	+	+
2.	Carbohydrates	1.Molish	+	-	-	-	+
		2.Fehling's	+	-	-	+	+
		3.Beneticts	+	-	-	+	-
3.	Proteins and Amino acids	1.Millions	+	-	-	+	-
		2.Killer Killiani	+	-	+	+	-
4.	Phytosterols	Libbermann's Burchard	+	+	+	+	+
5.	Oils	Spot Test	-	-	+	-	+
6.	Coumarin	Fluorescence	-	+	-	-	-
7.	Saponins	Foam Test	+	+	+	+	-
8.	Phenolic	1.Ferric Chloride	-	-	-	-	-
		2.Gelatin	-	-	-	-	-
9.	Glycosides and Flavonoids	1.Borntrager	-	-	-	-	-
		2.Lead Acetate	+	+	+	+	+
10.	Acid Reduction	Mg, Hcl Test	+	+	-	-	+

+ Present - Absent

4.2. Antimicrobial activity

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

4.2.1. Antibacterial activity of leaves extract of *Heliotropium indicum* L.

The leaves extracts were evaluated for its antibacterial activity against three clinical bacterial isolates and negative namely *Staphylococcus aureus*, *Shigella flexneri*, *Pseudomonas aeruginosa* and Dimethyl sulfoxide. Table.II described the antibacterial activity of ethanol, chloroform and petroleum ether extracts of the *Heliotropium indicum* L. against selected bacterial isolates. Fig. 4 shows the zone of inhibition of *Heliotropium indicum* L. against bacterial isolates.

The zone of inhibition was found to be maximum in the ethanol extract and also found to be more active against *Pseudomonas aeruginosa* (28mm), *Shigella flexneri* (25mm) *Staphylococcus aureus* (21mm) (Table II). The chloroform extract exhibited the zone of inhibition of 18mm, 15mm, 12mm against *Staphylococcus aureus*, *Shigella flexneri* and *Pseudomonas aeruginosa* respectively. The Petroleum ether extract exhibited the zone of inhibition of 10mm, 20mm, 25mm against *Staphylococcus aureus*, *Shigella* and *Pseudomonas aeruginosa* respectively.

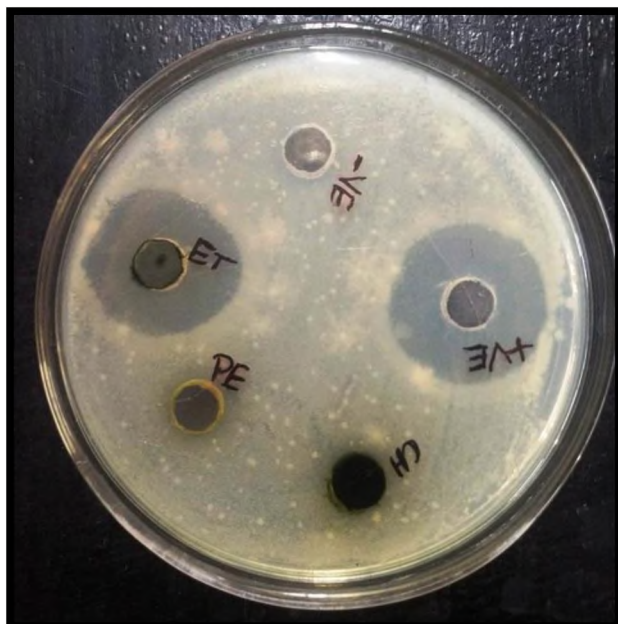
The Dimethyl sulfoxide extract exhibited the zone of inhibition of 16mm, 12mm, 13mm against *Staphylococcus aureus*, *Shigella flexneri* and *Pseudomonas aeruginosa* respectively. The positive control (Ampicilin exhibited the zone of inhibition of 36mm, 35mm, 30mm against *Staphylococcus aureus*, *Shigella flexneri* and *Pseudomonas aeruginosa* respectively)

Table II. Antibacterial activity of the leaves extracts of *Heliotropium indicum* L. against the bacterial isolates

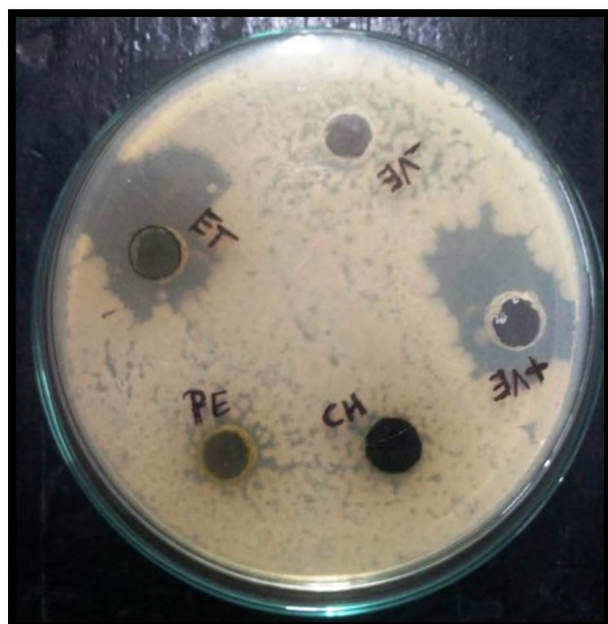
Bacterial Isolates	Zone of inhibition in diameter (mm)				
	EEHI	CHI	PE	NC DMSO	PC Ampicillin
<i>Pseudomonas aeruginosa</i>	26.5± 1.29	16.25 ± 1.5	24 ± 1.82	15 ± 2.16	33.25 ± 2.75
<i>Staphylococcus aureus</i>	22 ± 1.82	12.5 ± 1.29	10.25 ± 1.70	12.5 ± 2.08	33.25 ± 2.75
<i>Shigella flexneri</i>	19.75 ± 4.99	13 ± 2.16	18 ± 2.16	10.25 ± 1.70	28 ± 2.16

Values are expressed as mean ± standard deviation of the four replicates.

- EEHI - Ethanol extract of *H.indicum* L.
- CHI - Chloroform extract *H.indicum* L.
- PE - Petroleum extract *H.indicum* L.
- PC - Positive Control (Ampicillin)
- NC - Dimethyl sulfoxide



Shigella flexneri



Pseudomonas aeruginosa



Staphylococcus aureus

Fig.4. Antibacterial activity of *Heliotropium indicum* L. leaves extracts

- | | | |
|-----|---|--|
| ET | - | Ethanol extract of <i>H.indicum</i> L. |
| CH | - | Chloroform extract <i>H.indicum</i> L. |
| PE | - | Petroleum extract <i>H.indicum</i> L. |
| +VE | - | Positive Control (Ampicilin) |
| -VE | - | Dimethyl sulfoxide |

4.2.2. Antifungal activity of leaves extract of *Heliotropium*

indicum L.

The antifungal activity of the leaves extract of *Heliotropium indicum* L. was determined against the fungal isolates namely *Aspergillus niger* and *Rhizopus* species. Table.III depicts the antifungal activity of *Heliotropium indicum* L. leaves extracts. Fig. 5 shows the zone of inhibition of *Heliotropium indicum* L. against fungal isolates.

The ethanol extract of the *Heliotropium indicum* L. showed the maximum activity against *Rhizopus* (18mm) whereas the minimum activity was reported against and *Aspergillus niger* (13mm).

The Chloroform extract of *Heliotropium indicum* L. showed the maximum activity against the minimum activity against *Rhizopus* (17mm) followed by *Aspergillus niger* (15mm).

The aqueous extract exhibited the zone of inhibition of 14mm, 10mm against *Aspergillus niger*, *Rhizopus* respectively.

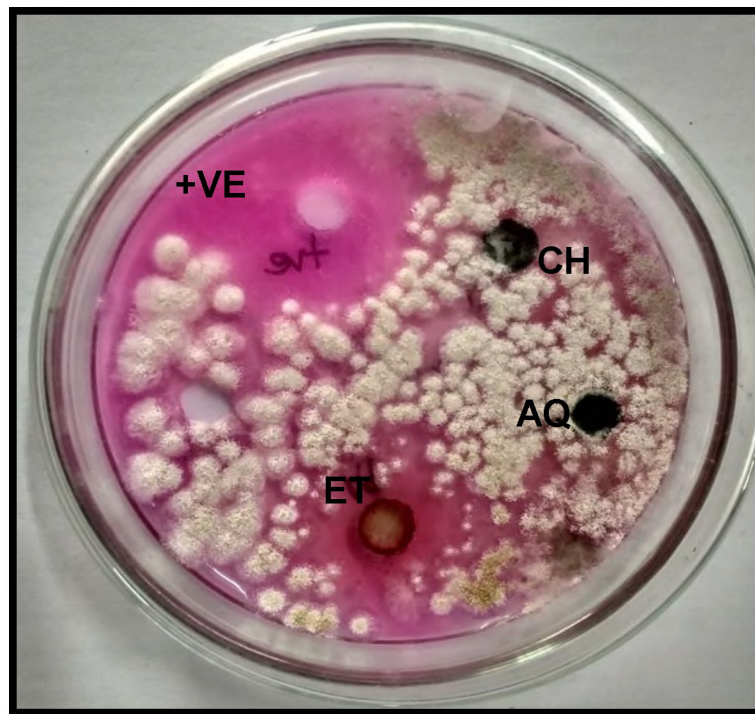
The inhibition zone for the tested fungi ranged from 10 - 19mm indicating a remarkable antifungal effect when compared with that of Fluconazole, the positive control, which ranged from 21 - 25mm.

Table III. Antifungal activity of the leaves extracts of *Heliotropium indicum* L. against the fungal isolates

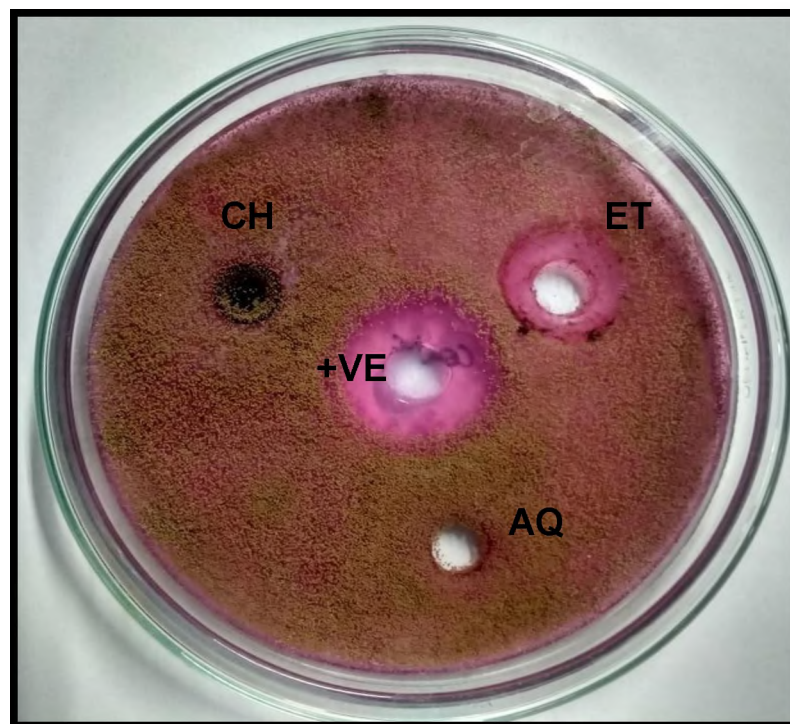
Fungal Isolates	Zone of Inhibition in Diameter (mm)			
	EEHI	CHI	AE	PC Fluconazole
<i>Aspergillus niger</i>	10.75 ± 1.70	12.25 ± 2.21	11.5 ± 2.38	13 ± 1.82
<i>Rhizopus</i>	16.5 ± 1.29	14.5 ± 3.10	9.5 ± 1.29	14 ± 3.16

Values are expressed as mean ± standard deviation of the three replicates.

- EEHI – Ethanol extract of *Heliotropium indicum* L.
- CHI – Chloroform extract *Heliotropium indicum* L.
- AE – Aqueous extract *Heliotropium indicum* L.
- PC – Positive Control (Fluconazole)



Rhizopus



Aspergillus niger

Fig.5. Antifungal activity of *Heliotropium indicum* L. leaves extracts

- | | | |
|-----|---|---|
| ET | – | Ethanol extract of <i>Heliotropium indicum</i> L. |
| CH | – | Chloroform extract <i>Heliotropium indicum</i> L. |
| AQ | – | Aqueous extract <i>Heliotropium indicum</i> L. |
| +VE | – | Positive Control (Fluconazole) |

4.3. Wound healing activity

4.3.1. General observations

No death was observed for any of the rats in the study groups and there were no remarkable changes in general appearance or animal behavior. The animals treated with *H.indicum* L. extracts had less scar formation at the wound sites and also had hair growing at the wound sites. The rats treated with anesthesia had prominent scars and no hair at the wound sites.

4.3.2. Wound contraction, complete epithelialisation and complete healing

The data pertaining to wound contraction on 21st day revealed 100% closure in treatment with EEHI with base followed by EEHI 98.3% which was on par with the standard drug 80.83%. EEHI with base extract had highest percentage wound closure followed by EEHI and then T-Bact ointment at day 4, 16 and 21 of treatment (Table IV). These time periods were significantly shorter than those for the controls at $p < 0.05$. EEHI with paraffin base demonstrated better cell differentiation and organization of the epithelial tissue at day 21 (Figure 8). Only EEHI with paraffin had a higher rate of healing than T-Bact ointment at day 21 (80.83 ± 1.23).

Table IV. Effect of ethanol extract on *Heliotropium indicum* L. on percent wound contraction and period

Treatment	Contraction of the wound (mm)				
	4 th day	8 th day	12 th day	16 th day	21 st day
Control	4.16 ± 3.87	11.83 ± 2.24	30.00 ± 1.23	50.00 ± 2.54	60.00 ± 1.23
T – Bact ointment (0.5% w/w)	5.08 ± 1.23	24.16 ± 1.54	53.33 ± 3.67	69.16 ± 3.30	80.83 ± 1.23
EEHI with base (200 mg/kg)	7.50 ± 0.14	30.83 ± 0.23	63.33 ± 0.64	92.50 ± 0.12	100.00 ± 0.00
EEHI Extract (200 mg/kg)	5.90 ± 0.13	27.00 ± 2.31	59.00 ± 1.23	75.00 ± 0.32	98.33 ± 1.32
SE	0.831	0.710	0.622	0.582	0.520
F Test	**	**	**	**	**

P<0.05 when compared with control group

EEHI *with base* – Ethanol extract of *H.indicum* L. with paraffin wax

EEHI *Extract* – Ethanol extract of *H.indicum* L.

T – bact ointment – Positive control

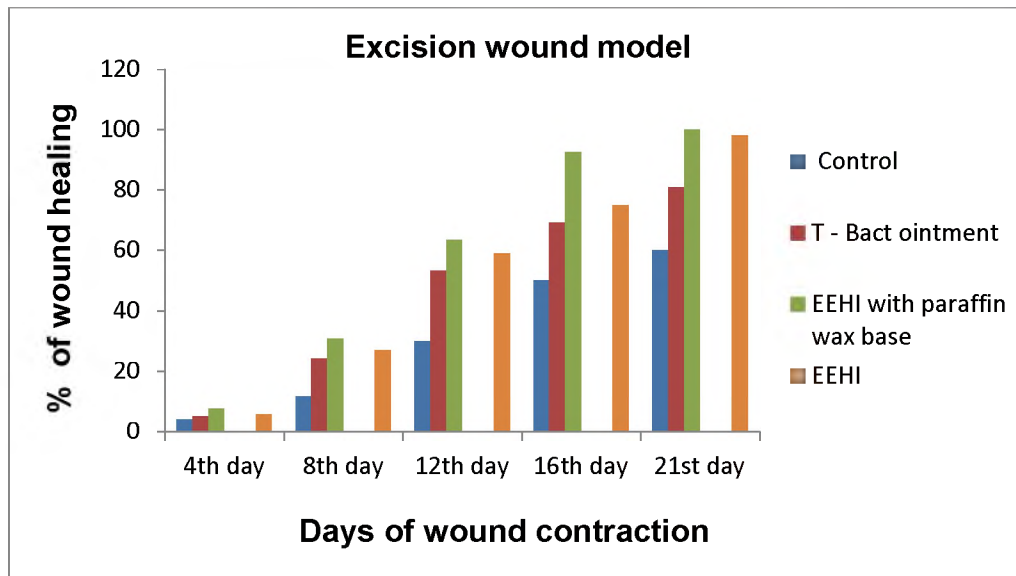


Fig.6. Effect of EEHI on wound contraction

- EEHI with base (200mg) – Ethanol extract of *H.indicum* L. with paraffin wax
- EEHI Extract (200mg) – Ethanol extract of *H.indicum* L.
- T – Bact ointment (0.5% w/w) – Positive Control

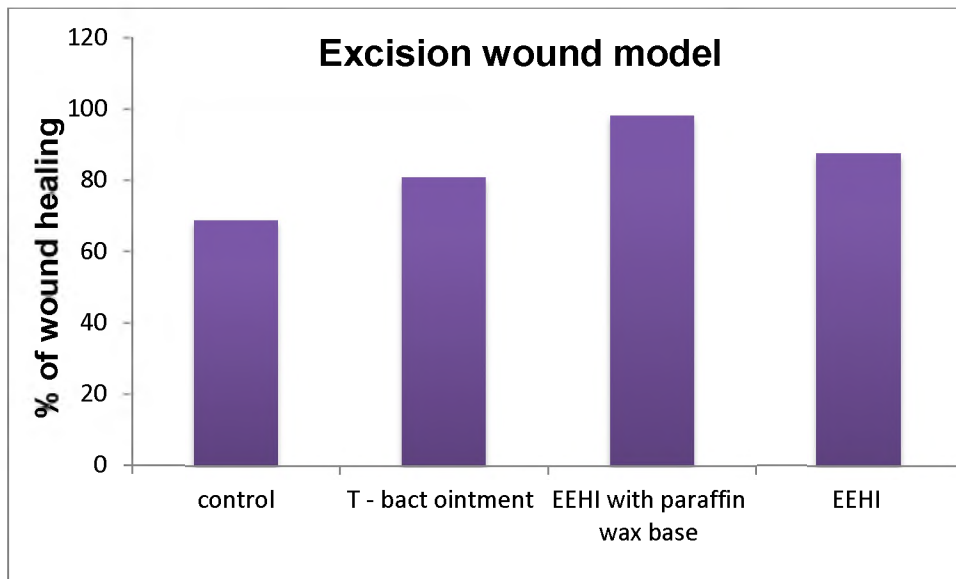


Fig.7.Effect of EEHI on wound contraction

- EEHI with base (200mg) – Ethanol extract of *H.indicum* L. with paraffin wax
- EEHI Extract (200mg) – Ethanol extract of *H.indicum* L.
- T – Bact ointment (0.5% w/w) – Positive Control

Table. V Effect of ethanol extract on *Heliotropium indicum* L. on epithelialisation period

Treatment	Epithelialisation (Days)
Control	21
T – bact ointment	17
EEHI with base	12
EEHI Extract	15

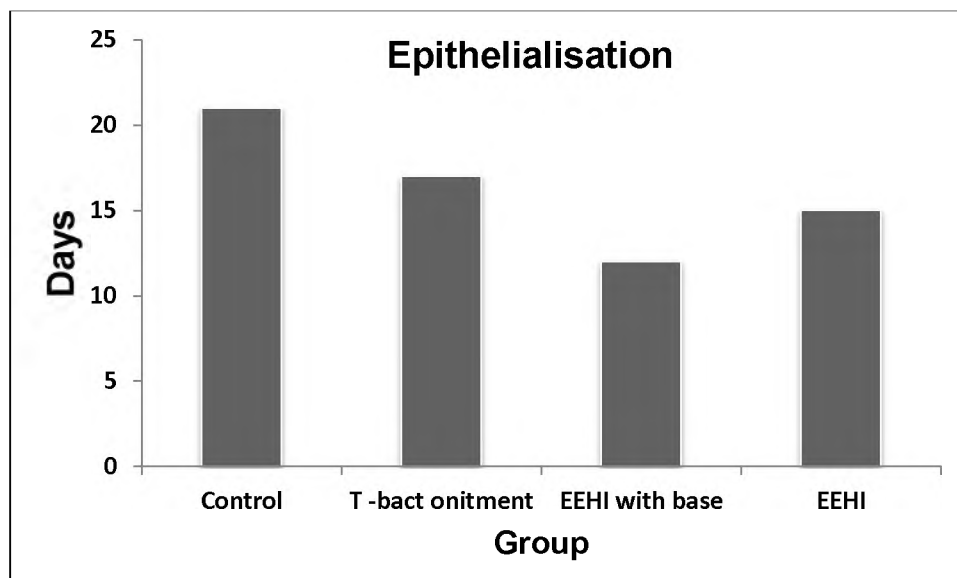


Fig.8.Effect of EEHI on epithelialisation

- EEHI with base (200mg) – Ethanol extract of *H.indicum* L. with paraffin wax
- EEHI Extract (200mg) – Ethanol extract of *H.indicum* L.
- T – Bact ointment (0.5% w/w) – Positive Control

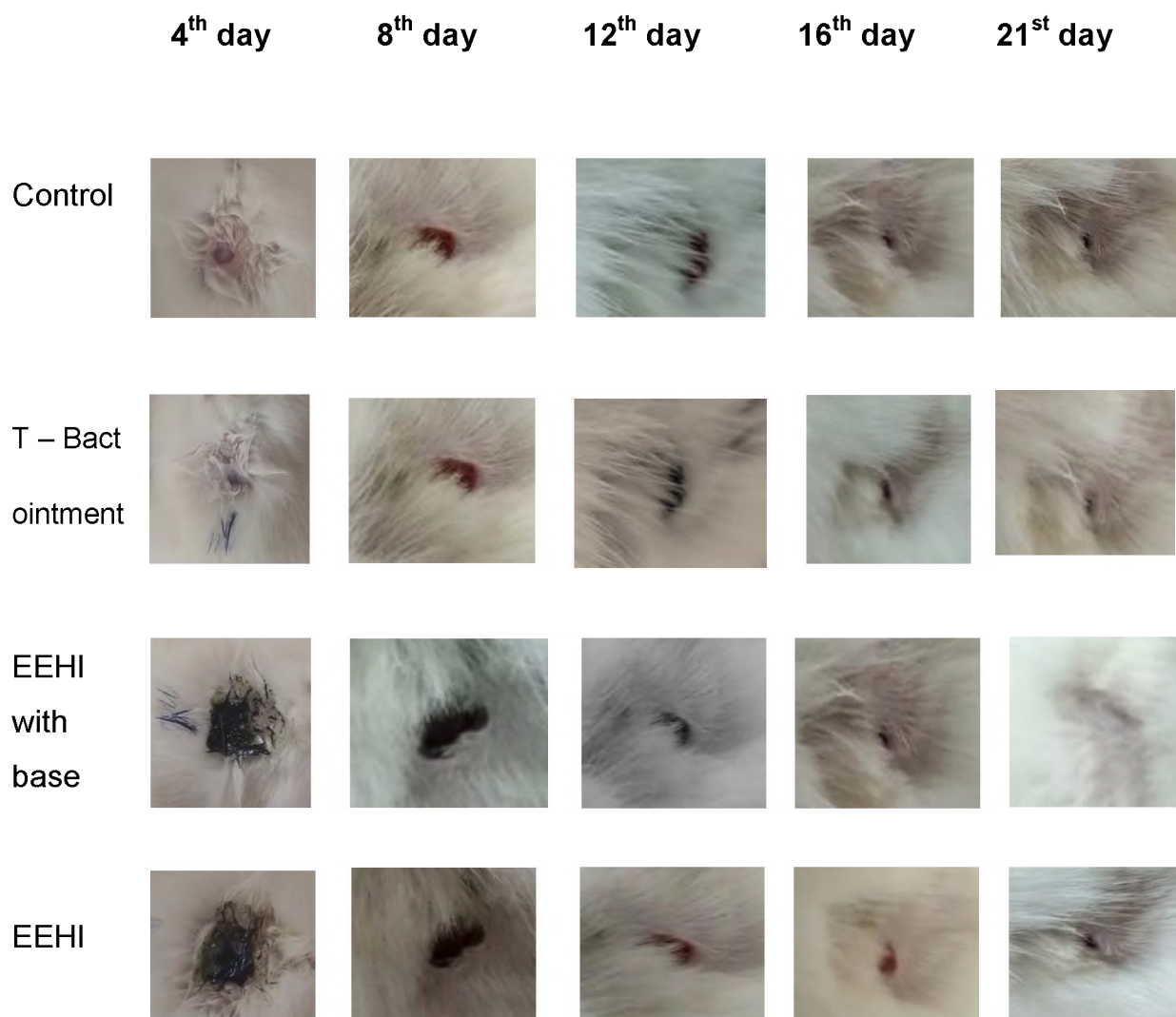


Fig.9. Wound areas of different groups in excision wound model

- EEHI with base (200mg) – Ethanol extract of *H.indicum* L. with paraffin wax
- EEHI Extract (200mg) – Ethanol extract of *H.indicum* L.
- T – Bact ointment (0.5% w/w) – Positive Control

V DISCUSSION

Use of leaves as a source of medicine has been inherited and is an important component of the health care system in India. Leaves extracts are given singly or as concoctions for various ailments (Ahmad, *et al.*,2014). Many investigations have demonstrated to elucidate the chemical compounds of leaf origin. In the present study, phytochemical, antimicrobial and wound healing activities of the leaf extracts was carried out to find out the major bioactive present in the leaves extracts (Dash and Murthy,2014).

The phytochemical screening and qualitative estimation of the plant studies showed that the leaves were rich in alkaloids, flavonoids, phenols in all the extracts. Some extract showed presence of carbohydrates and sterols too. Saponins and tannins were found to be present in almost all the extracts of the extract (Bruno, *et al.*,1991).

Flavonoids are polyphenolic compounds and consist of flavones, flavonols, flavanols, flavanone and flavanonols. These compounds represent the majority of plant secondary metabolites and have shown to possess remarkable health promontory effects such as anti-inflammatory, antioxidant, antimicrobial, anticancer properties. Interception of free radicals or other reactive species is mainly by radical scavenging and is caused by various antioxidants like vitamin C and E, glutathione, other thiol compounds, carotenoids, flavonoids (Deba, *et al.*,2008)

All the plant extracts used in this study were primarily screened against the test microorganisms by different methods like agar well diffusion and disc diffusion and the determination of minimum inhibitory concentrations (Getie, *et al.*,2002). According to the World Health Organization (2012) the evolving public health threat of antimicrobial resistance is driven by both appropriate and inappropriate use of anti-infective medicines. The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. Different antibiotics exercise their inhibitory activity on different pathogenic organisms (Hashemnia, *et al.*,2001).

The present study was undertaken to evaluate whether *Heliotropium indicum* L. leaves promote wound healing in experimentally induced wounds in rats. The results of the present study substantiate the use of *Heliotropium indicum* L. (Dash and Murthy,2011) leaves in folklore medicine for the treatment of wounds. The emulsifying base containing hydro alcoholic extract applied topically promoted the breaking strength, wound contraction and period of epithelization in different models of experimental wounds. Collagenation, wound contraction and epithelization are crucial phases of wound healing (Esra, *et al.*,2009)

The phases of inflammation, macrophasia, fibroplasia and collagenation are intimately interlinked. Thus an intervention into any one of these phases by drugs could eventually either promote or depress one, other or all phases of healing (Frank and Kämpfer,2003). Growth hormone is known to promote the healing process by enhancing epithelial cell proliferation and cell collagen formation. Collagen is the family of protein, which provide structural support and it is the main component of tissue such as fibrous tissue, cartilage. The collagen synthesis is stimulated by various growth factors (Kamath and Rana,2003).

Growth hormone is also known to promote the proliferation of fibroblasts and fibroblast proliferation form the granulation tissue. The exact mechanism by which *Heliotropium indicum* L. leaves increased the granuloma tissue weight and breaking strength of granulation tissue cannot be explained with the present data. Lipid peroxidation is an important process of several types of injuries like burn, inflicted wound and skin ulcers. A drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils, increasing the strength of collagen fibers by an increase in circulation, thereby preventing the cell damage and promoting the DNA synthesis.

Several antioxidants such as vitamin C, metronidazole and vitamin E are reported to increase the wound healing (Khan, *et al.*, 2012). The wound healing activity of *Heliotropium indicum* L. leaves extract can be attributed to the presence of their phyto constituents which may be active individually or it

could be a synergistic activity of these constituents. It is speculated that tannins by virtue of its anti oxidant, anti inflammatory, astringent, and antimicrobial properties could be one of the contributors for the wound healing effect of the plant (Kumarasamyraja, *et al.*, 2012).

Moreover, antioxidant nature of sterols and the flavonoids of *Heliotropium indicum* L. could also be responsible for wound healing activity. From the results of this study it can be concluded that the hydro alcoholic extract of *Heliotropium indicum* L. has significant wound healing activity in excision models Lodhi, *et al.*, 2006,

Regarding contraction rate of neem oil on full thickness cutaneous wounds, neem oil almost showed a statistically non-significant difference throughout and at the end of study period as compared to Oxoferin. Significant effect on wound contraction rate of neem oil might be due to its neovascularization effect of neem oil (*Azadirachta indica*), which is considered as one of important factors for wound healing as reported by Emeka *et al.*, 2002.

Healing time of neem oil treated wounds exhibited statistically non-significant difference with that of Oxoferin. Good results regarding healing time were matched with the findings of Somashekar *et al.*, 2010 and Sunil *et al.*, 2009 They reported that good results in wound healing by topical application of neem oil (*Azadirachta indica*) might be due to its herbal preparation possessing antiseptic, anti inflammatory and anti oxidant properties.

In this study EEHI plant extract has also significant results when compare too many models of wound contraction. The antibacterial and in vivo efficacy of aqueous fraction of *M. oleifera* in promoting wound healing in an animal model of diabetes. The antibacterial properties exhibited by aqueous fraction of *M. oleifera* may be linked to the presence of some phytochemical compounds, such as alkaloids, triterpenoids, tannins, and flavonoids, in the extracts reported in our earlier study. These compounds are known to promote the wound healing process mainly due to their astringent and antimicrobial

properties, which are responsible for wound contraction and increased rate of Muhammad *et al.*, 2015; Nayak *et.al.*,2006.

In the present study, in excision wound model, both the extracts showed faster healing when compared with control group. Significant reduction in wound size and period of epithelization as well as increase in percentage wound contraction was noticed in the extract treated animals. Results of histopathological studies carried out by Kumar *et al.*, 2001 also substantiates the wound healing activity revealed by the excision wound model.

In excision wound healing model the ethanol extract of the leaves of the plant *Heliotropium indicum* L. showed significant increase in percentage closure. This enhancement may be due to the effect of *Heliotropium indicum* L. extracts on enhanced collagen synthesis. The higher breaking strength indicates better healing of wounds. Thus it supports the wound healing activity of *Heliotropium indicum* L. (Shukla, *et al.*,1998).

To conclude, leaves of *Heliotropium indicum* L. possess good wound healing activity when applied locally. The high dose of the extract was found to be more effective topically than the low dose. Further, isolation of active constituents from the extracts of the leaves may bring about the development of a new wound-healing agent.

VI SUMMARY AND CONCLUSION

The therapeutic effects of medicinal plants for the treatment of various diseases are based on the chemical compounds of these plants (Shetty, *et al.*, 2008) The major components are organic compounds, some of which have biological activity, but none act independently and cannot replace the functions of the medicinal plant as a whole.

Phytochemical analyses revealed that ethanol extracts of *Heliotropium indicum* L. contain terpenoids, alkaloids and flavonoids which have been reported to be responsible for the antimicrobial properties and, it could serve as antimicrobial agents for the treatment of microbial infections (Scortichini and Rossi, 1991). Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects. They exhibit marked physiological activity when administered to animals.

Flavonoids on the other hand are potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity (Rahman, *et al.*, 2011). Flavonoids in intestinal tract lower the risk of heart disease. As antioxidants, flavonoids from candidate plants provide anti-inflammatory activity. This may be the reason *Heliotropium indicum* L. would be suggested for an alternative in herbal medicine (Harborne, *et al.*, 1990).

The development of antibiotic resistance is multi factorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors (Pattanayak and Sunita, 2008). This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs (Prasanta, 2013).

Generally microbes have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents. One way to prevent antibiotic resistances is by using new compounds which are not based on the existing synthetic antimicrobial agents (Rojas, *et al.*,2006). These antimicrobial substances are of natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents.

However, some medicinal herbs for some reasons have not found wider application and sometimes are referred as „forgotten plants“ such as *Heliotropium indicum* L. (Dash and Murthy, 2011). Taking this into account it is reasonable to revise the „forgotten plants“ by assessing their applicability and benefits using modern scientific analysis methods.

The result obtained from the study points out that the active component present in ethanol extract of *Heliotropium indicum* L. can prove to be a great remedy for treating diseases. The zone of inhibition for the tested microorganism, such as bacteria and fungi indicating a remarkable antibacterial effect when compared with the positive control (Tsuchiya and sato,1996).

H. indicum L. is very rich in pyrrolizidine alkaloids. Numerous pyrrolizidine alkaloids have been identified in this plant by several authors (Vinothapooshan and suntar,2010; Yeo, *et al.*,2011). The alkaloids reported in the entire plant include heliotrine, lasiocarpine, indicine, 12-acetyl indicine, indicinine, indicine-N-oxide, retronecine, trachelan-thamide with traces of supinidine and lindelofidine. The aerial parts contain echinatine, heleurine, lasiocarpine-N-oxide, supinine, heliotrine, indicine, indicine-N-oxide and lasiocarpine.

The present study demonstrated that the ethanol extract of *Heliotropium indicum* L. have properties of promoting accelerated wound-healing activity compared with control. The study provides scientific evidence for further evaluation of *Heliotropium indicum* L. in the topical treatment and management of wounds.

REFERENCES

- Abajo, C., Boffill, M.A., Campo, J.D., Mendez, M.A., Gonzalez, Y., Mitjans, M. and Vinardell, M.P.2004. *In vitro* study of the antioxidant and immune modulatory activity of aqueous infusion of *Bidens pilosa*. *J. Ethno pharmacol.*, 93: 319-323.
- Abdulla, M.A., Hassandarvish, A. and Hapipa, M.A.2009. Acceleration of wound healing potential of *Lantana camara* leaf extract in experimental rats. *Res. J. Med. Sci.*, 3(2): 75-79.
- AbduRahim, S.A., Mohammed, N.B. and Almagboul, A.Z.2016. *In vivo* antibacterial and wound healing activities of *acacia ehrenbergiana*, *Mediterranean Journal of Biosciences* ,1(4): 147-152.
- Abiramasundari, P., Priya ,V., Jeyanthi,G.P. and GayathriDevi,S.2011. Evaluation of the antibacterial activity of *Cocculus hirsutus* . *Hygeia - Journal for Drugs and Medicines*, 13 (2): 26-31.
- Abraham, A., Reinu E., Cynthia ,S., Wong and Munish Puri.2016. Enrichment of cellulosic waste hemp (*Cannabis sativa* hurd into non-toxic microfibres. *Materials*, 9: 562.
- Ahmad, S., Ahmad, S., Bibi, A., Ishaq, M.S., Afridi, M.S., Kanwal, F., Zakir, M. and Fatima, F.2014. Phytochemical analysis, antioxidant activity, fatty acids composition, and functional group analysis of *Heliotropium bacciferum*.
- Akkol ,E.K., Koca, U., Pesin, I. and Demet, Y.2009. Evaluation of the wound healing potential of *Achillea biebersteinii* Afan. *Asteraceae* by *in vivo* excision and incision models.
- Akram, E., Masoud, K. and Vahid A.2012 Evaluation of the antibacterial and wound healing activity of *Quercus persica*. *J Basic App Sci* 8: 118-123.
- Alam, G., Singh, M. and Singh, A. wound healing potential of some medicinal plants. *Int. J. Pharm. Sci. Rev. Res.* 2011, July; 9(1):136.

- Anonymous, The Indian Pharmacopoeia, 2 nd ed., Manager of Publications, Govt. of India, Ministry of Health & Family Welfare, Delhi,2003.(1966), 267, 656.
- Anonymous, The Wealth of India, Publication and Information Directorate, CSIR, New Delhi, 204.
- Ashafa,T. and Afolayan, A.J. 2009. Screening the root extracts from *Biden pilosa* L. var. *Radiata* (Asteraceae for antimicrobial potentials). *J. Med. Plants Res.*, 3(8): 568-572.
- Azadil, H.G., Fathi, B., Mehrjerdi, H.K., Maleki, M., Shaterzadeh, H. and Abyazi , M.2011. Macroscopic evaluation of wound healing activity of the persian shallot, *Allium hirtifolium* in rat, *Iranian Journal of Veterinary Science and Technology* ,3, (1): 31-38.
- Balandrin, M.F., Klocke, J.A., Wurtele, E.S. and Bollinger, W.H., 1985. Natural plant chemicals: sources of industrial and medicinal materials. *Science* 228, 1154–1160.
- Balandrin, M.F.1985. *Science*, 228, 1154.
- Bartkova,J., Grøn, B., Dabelsteen, E. and Bartek J. Cell-cycle regulatory proteins in human wound healing. *Archives of oral biology*, 48(2): 2003, 125-132.
- Basak, M., Biplab, K.R. and Dey. 2016. *PHYTOCHEMICAL AND ANTIPYRETIC POTENTIAL OF ETHANOLIC LEAF EXTRACT OF HELIOTROPIUM INDICUM SPECIES. Journal of Applied Pharmaceutical Research*, 4 (2): 06 – 10.
- Bharti, M. and Saxena, R.C., Baghel, O.S., Saxena, R. and Apte, K.G.2011. wound healing activity of leaf of *Nyctanthes arbor-trisitis*, *IJPSR*; 2: 2694-98.
- Bohlmann, F., Wallmeyer, M., King, R.M. and Robinson, H.1984. 2-Oxolabda-8(17):13-dien-15-ol from *Ophryosporus chilca*. *Phytochemistry* 23, 1513–1514.

- Boominathan, R., Parimaladevi, B., Mandal, S.C. and Ghoshal, S.K.2004 Anti-inflammatory evaluation of *Ionidium suffruticosam* Ging in rats, *J. Ethnopharmacol.*, 91 (2-3): 367-70.
- Brack, A.1999. Diccionario enciclopédico de plantas utiles del Perú. CBC, Cuzco, Perú, 550.
- Bruno, M., Savona, G., Piozzi, F., de la Torre, M.C., Rodriguez, B. and Marlier, M.1991. Abietane diterpenoids from *Lepechinia meyenii* and *Lepechinia hastata*. *phytochemistry* 38, 2339–2343.
- Bustamante, B. and Campos, P.E.2001. Endemic sporotrichosis. *Current opinions in infectious diseases* 14, 145–149.
- Carolina, E., Carlos, A., David, F., Ike, M.S. and David, R. 2015. The influence of growth factors on skin wound healing in rats, *Braz J Otorhinolaryngol.* 82(5):512-521.
- Chadha, Y.R. *The Wealth of India*, C. S. I.R., New Delhi, India, 1985.
- Chew, A.L., Jessica, J.J.A. and Sasidharan, S.2012. Antioxidant and antibacterial activity of different parts of *Leucas aspera*, *Asian Pac J Trop Biomed* 2(3): 176-180.
- Das.P, Palai,S.K. and Samantaray,S.1999 *In vitro* somatic embryogenesis in *Typhonium trilobatum* schott. *Plant Growth Regulation* 27: 193-97.
- Dash, G.K. and Murthy, P.N. 2011 Studies on wound healing activity of *Heliotropium indicum* leaves on Rats. *International Journal of Scholarly Research Network* 2011; 1: 1-8.
- Deba, F., Xuan, T.D., Yasuda, M. and Tawata S.2008. Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. Var. *Radiata*. *Food. Control*, 19: 346-352.
- Dewangan, H., Bais, M., Jaiswal, V. and Vinod Kumar Verma.2012 potential wound healing activity of the ethanolic extract of *Solanum xanthocarpum schrad* and *wendl* leaves. *Pak. J. Pharm. Sci.*; 25: 189-194.

- Dhaliwal, G.S. and Arora, R.1998 Principles of insect pest management, Kalyani Publishers, Ludhiana, 107.
- Diener, W. and Mischke, U. *et al.*, 1995. "The evaluation of the OECD modified version of acute toxicity class method oral" Arch. Toxicol.,69: 729-734.
- Duke, J.A. and Vasquez, R.1994. Amazonian Ethnobotanical Dictionary. CRC Press, Boca Raton, 215.
- Ehrlich, H.P. and Hunt, T.K.1969 "The effects of cortisone and anabolic steroids on the tensile strength of healing wounds", *Annals of Surgery*,170 (2):203-206.
- El-Seedi, H.R., Ohara, T., Sata, N. and Nishiyama, S.. 2002. Antimicrobial diterpenoids from *Eupatorium glutinosum* (Asteraceae). *Journal of Ethnopharmacology* 81, 293–296.
- Esra, A., Koca, S., Demet, A., Gulnur, J. and Yesilada, E.2009. Exploring the wound healing activity of *Arnebia densiflora* (Nordm. Ledeb) by *in vivo* models, *Journal of Ethanopharmacology* 124,137-141.
- Feo, D.V.1992. Medicinal and magical plants in the northern *Peruvian Andes*. *Fitoterapia* 63, 417–440.
- Finegold, S.M. and Martin, W.J., 1982. Diagnostic Microbiology. The C.V. Mosby Company, St. Louis,81–91, 158–168, 287–290.
- Frank, S. and Kämpfer, H.2003 Wound healing method and protocols. New Jersey: Humana Press Inc; 3.
- Gardner, S.E., Frantz, R.A., Saltzman, C.L. and Dodgson, K.J.2004. *Staphylococcus aureus* is associated with high microbial load in chronic wounds. *Wounds*, 16(8):
- Geissberger, P. and Sequin, U. 1991. Constituents of *Bidens pilosa* L – Do the components found so far explain the use of this plant in traditional medicine? *Acta. Trop.*, 48(4): 251-261.

- Getie, M. and Mariam, G.T. *et al*, 2002 “Evaluation of the release profiles of flavonoids from topical formulations of the crude extract of the leaves of *Dodonea viscosa* (Sapindaceae”, *Pharmazie*), 57 (5): 320-322.
- Ghashghaii, A., Hashemnia, M., Nikousefat, Z., Mahdi, M., Zangeneh, A. and Zangeneh, A. 2017. *J. Pharmaceutical Sciences.*, 23, 256-263.
- Goyal, N., Surendra, K.R. and Sharma. 2014. Bioactive phytoconstituents and plant extracts from genus *Heliotropium*. Department of pharmaceutical sciences, Roorkee college of pharmacy, Roorkee, Uttarakhand.
- Hamburger, M. and Hostettmann, K. 1991. Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry* 30, 3864–3874.
- Hammond, G.B., Fernández, I.D., Villegas, L.F. and Vaisberg, A.J., 1998. A survey of traditional medicinal plants from the Callejón de Huaylas, Department of Ancash, *Perú*. *Journal of Ethnopharmacology* 61, 17–30.
- Harborn, J.B., *Phytochemical Methods*, Chapman and Hall, London, 1998, 90-203.
- Harborne, J.B. 1990. Role of secondary metabolites in chemical defence mechanisms in plants. *Bioactive compounds from plants*. Ciba Foundation Symposium 154. Wiley, Chichester, 126–139.
- Hoog, D., Guarro, G.S., Gené, J. and Figueras, J. 2000. *Atlas of Clinical Fungi*. Central bureau voor Schimmel cultures, Utrecht, The Netherlands/University Rovira i Virgili, Reus, Spain, 755–757, 925–927.
- Ilango, K. and Chitra, V. Wound healing and anti-oxidant activities of the fruit pulp of *Limonia Acidissima* L. *Rutaceae* in Rats. *Tropical Journal of Pharmaceutical Research* June 2010; 9 (3): 223-230.
- Jacobson, M. 1975 *Agricultural hand Book*, US Department of Agriculture, Washington D.C., 138.

- Janssen, A.M., Scheffer, J.J., Ntezurubanza, L. and Baerheim, S.A. 1989. Antimicrobial activities of some *Ocimum* species grown in Rwanda. *J. Ethnopharmacol.*, 26(1): 57-63.
- Jayasutha, J, Josephine., J.M. and Nithila, S. Evaluation of wound healing activity of ethanolic extract of *Aristolochia bracteata* and *Cassia tora* on Wistar Albino rats. *Int J Pharm Tech Res* 2011; 3: 1547-50.
- Kalyon, R., Shivakumar, H. and Sibaji, S. 2009. Wound healing potential of leaf extracts of *Ficus Religiosa* on wistar albino strain rats. *Int. J.Pharm. Tech. Res.*, 1(3): 506-508.
- Kamath, J.V. and Rana, A.C.2003. *et al*, "Prohealing effect of *Cinnamomum zeylanicum* bark", *Phytotherapy Research*, 17(8): 970-972.
- Kamath, J.V., Rana, A.C. and Chowdhury, A. "Prohealing effect of *Cinnamomum zeylanicum* bark," *Phytotherapy Research*, 17, (8) 970–972, 2003.
- Kamboj, A., Kumar, S. and Kumar, V.2013. Evaluation of anti diabetic activity of hydro alcoholic extract of *Cestrum nocturnum* leaves in streptozotocin-induced diabetic rats. *Adv. Pharmacol. Sci*,4.
- Khan, M.A., Saxena, A., Fatima, F.T., Sharma, F.G., Goud, V. and Husain, A.2012. Study of wound healing activity of *Delonix regia* flowers in experimental animal models. *Am. J. PharmTech Res*; 2: 380-90.
- Kiran, K. and Asad, M.2008 wound healing activity of *Sesamum indicum* L. seed and oil in rats. *Indian Journal of Experimental Biology*, 46, 777-782.
- Kirtikar, K.R. and Basu, B.D.1994. *Indian Medicinal Plants: Bishen Singh Mahendrapal Singh*, Klewer, Dehradun, India.
- Kirtikar, K.R. and Basu, B.D.1999 Indian medicinal plants, III, International Book Distributors, Dehra Dun, 1999, 2155-2156.

- Kumar, B., Vijayakumar, M., Govindarajan, R. and Pushpangadan, P. 2007 "Ethnopharmacological approaches to wound healing exploring medicinal plants of India," *Journal of Ethnopharmacology*, 114, (2): 103- 113.
- Kumarasamyraja, D., Jeganathan, N.S. and Manavalan, R. 2012 A Review on medicinal plants with potential wound healing activity. *International Journal of Pharma Sciences*. 2, (4): 101-107.
- Kyakulaga .A. Hassan, Deogratius, O., Jane, F., Nyafuono, A., Francis, O. and Ogwang, P. 2011. Wound healing potential of the ethanolic extracts of *Bidens pilosa* and *Ocimum suave*, *African journal of pharmacy and pharmacology*.
- Levine, S. 1970. "The effect of povidone-iodine in controlling skin flora beneath occlusive dressings", *Journal of the American Podiatry Association*, 60(12): 486–487.
- Lodhi, S., Pawar, R., Jain, A. and Singhai, A. 2006. Wound healing potential of *Tephrosia purpurea* Linn. *Pers. in rats. J. Ethnopharmacol* 108: 204-10.
- MacSween, R.N. and Whaley, K. 1995. *Muir's Text Book of Pathology*. 13th edition. London : Arnold:158.
- Mahato, K., Kakoti, B.B., Borah, S. and Kumar, M. 2015, *In-vitro* anti oxidative potential of methanol aerial extracts from three ethno medicinal plants of Assam: A comparative study. *Journal of Applied Pharmaceutical Science* 5 (12):111-116
- Malviya, N. and Jain, S. 2009. Wound healing activity of aqueous extract of radix *paeoniae* root. *Acta poloniae Pharmaceutica ñ Drug Research*, 66: 543-47.
- Midwood, K.S., Williams, L.V. and Schwarzbauer, J.E. 2004. Tissue repair and the dynamics of the extracellular matrix. *Int. J. Biochem. Cell. Biol. Jun*; 36(6): 1031-7.

- Mikhail, E.V., Ivanova, A.V., Anurov, M.V. and Titkova, S.M. 2003, 2004. Wound healing effect of papaya-based preparation in experimental thermal trauma. *Bulletin of experimental biology and medicine*, 137, (6): 638-640.
- Morton, J.J. and Malone, M.H. 1972. "Evaluation of vulnerary activity by an open wound procedure in rats", *Archives Internationales de Pharmacodynamic et de Therapie*, 196 (1): 117-126.
- MOT, A., SILAGHI-DUMITRESCU, R., SÂRBU, C. 2011. "Rapid and effective evaluation of the antioxidant capacity of propolis extracts using DPPH bleaching kinetic profiles, FT - IR and UV - vis spectroscopic data", *Journal of Food Composition and Analysis*, 24, 4-5, 516-522.
- Muhammad, A.A., Arulsevan, P., Cheah, P.S., Abas, F. and Fakurazi, S. 2016. Evaluation of wound healing properties of bioactive aqueous fraction from *Moringa oleifera* Lam on experimentally induced diabetic animal model, *Drug Design, Development and therapy*.
- Muhammad, H.S. and Muhammad, S. 2005. "The use of *Lawsonia inermis* linn. *henna* in the management of burn wound infections." *African Journal of Biotechnology*, 4 (9): 934-937.
- Murti, K. and Kumar, U. 2012. Enhancement of wound healing with roots of *Ficus racimosa* L. in albino rats, *Asian Pacific Journal of Tropical Biomedicine*. 2: 276-80.
- Nayak, B.S. and Lexley, M. and Pereira, P. 2006 *Catharanthus roseus* flower extract has wound-healing activity in Sprague Dawley rats. *BMC complementary and alternative medicine*. *BMC complementary and alternative medicine* 6: 1-6.
- Nayak, S.B., Sandiford, S. and Maxwell, A. 2007. Evaluation of the wound healing activity of ethanolic extract of *Morinda citrifolia* L. leaf. *eCAM*, 4: 1-6.

- Nitin, K., Upadhyay, R., Kumar, R., Siddiqui, M.S. and Gupta, A.2009. Mechanism of wound healing activity of *Hippophae rhamnoids L.* leaf extract in experimental burns. Evidence - based complementary and alternative medicine, 2011. Article ID 659705.
- Paarakh., P.M., Chansouria, J. P. N. and Khosa, L. Wound healing activity of *Annona muricata* extract. *J Pharm Res* 2009; 2: 404-406.
- Panda, V., Sonkamble, M. and Patil, S.2011. Wound healing activity of *Ipomoea batatas* tubers sweet potato. *Functional foods in health and disease*, 10: 403-15.
- Pattanayak, S.P. and Sunita, P.2008. Wound healing, anti-microbial and antioxidant potential of *Dendrophthoe falcate* L.f. Ettingsh. *J Ethnopharmacol* 120: 241-247.
- Prasanta, K., Ghosh, R. and Gaba, A.2013. Phyto-extracts in wound healing, *J Pharm Pharm Sci* , 16(5): 760 – 820.
- Rahman, A., Abd-Ellatif, S., Deraz, S. and Khalil, A. 2011. Antibacterial activity of some wild medicinal plants collected from western Mediterranean coast, Egypt: Natural alternatives for infectious disease treatment. *African Journal of Biotechnology*, 10(52): 10733-10743.
- Rahman, M.A., Mia, M.A. and Shahid, Z.2011.Pharmacological and phytochemical screen activities of roots of *Heliotropium indicum* *Pharmacology online* 1: 185-192.
- Rahmatullah, M., Rahman, L., Rehana, F., Kalpana, M.A. and Khatun, M.A., *et al.*,2010. A Scientific Evaluation of medicinal plants used in the folk medicinal system of five villages in Narsinghdi District, Bangladesh. *American- Eurasian Journal of Sustainable Agriculture*; 4: 55-64.
- Raina, R., Prawez, S., Verma, P.K. and Pankaj, N.K. 2008. Medicinal plants and their role in wound healing. *Vet Scan*, 3(1): 1-7.

- Rajabi, M.A. and Rajabi, F.2007. Effect of estrogen of wound healing in rats. *Pak. J. Med. Sci.*, 23(3): 349-352.
- Rajan, T.S., Kumar, T.R., Kumar, E.U., Sekar, M. and Senthil, M.K.2011. Antidiabetic activity of methanolic extract of *hibiscus cannabinus* in streptozotocin induced diabetic rats *Int. J. Pharma. Bio Sci.* 2(1): 125-130.
- Reddy, J.S. and Rao, P.R., *et al.*,2002 “Wound healing effects of *Heliotropium indicum*, *Plumbago zeylanicum* and *Acalypha indica* in rats” *Journal of Ethnopharmacology*, 79(2): 249-251.
- Rojas, J.J., Ochoa, V.J, Ocampo, S.A. and Munoz, J.F.2006. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of nonnosocomial infections. *BMC complement. altern. Med.*, 6: 2.
- Sambrook, J. and Russell, D.W.2011 In: *Molecular Cloning: A laboratory manual*, 3rd edition, cold spring harbor laboratory press. NY,7.4-7.8.
- Scortichini, M. and Rossi, M.P.1991. “Preliminary in vitro evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora* Burrill,” *Journal of Applied Bacteriology*, 71 (2):109-112.
- Shantha, N.S. and Napolitano, G.E.1992. “Gas chromatography of fatty acids,” *Journal of Chromatography*, 624, 1-2, 37– 51.
- Sharma, R.K., Rajni, G.P., Nathiya, D. and Sharma, A.K.2015. Assessment of wound healing activity of roots of *Bauhinia variegata* Linn. by excision and incision model in Albino Rats. *Asian Journal of Research in Pharmaceutical Science*, 5.
- Sharma, S., Sharmaa, M.C. and Kohli, D.V.2010. Wound healing activity and formulation of Ether-Benzene-95% ethanol extract of herbal drug *Madhuca Longifolia* leaves in Albino rats. *J Optoelectr Biomed Mat*,1: 13 – 15.

- Shenoy, C., Patil, M.B., Kumar, R. and Patil, R. 2009 Preliminary phytochemical investigation and wound healing activity of *allium cepa* L. (*liliaceae*). *International journal of pharmacy and Pharmaceutical Sciences* 2: 167-75.
- Shetty, S., Udupa S. and Udupa, L. 2008. Evaluation of antioxidant and wound healing effects of alcoholic and aqueous extract of *Ocimum sanctum* linn in rats. *Evid. Based. Complement. Altern. Med.*, 5: 95-101.
- Shukla, A., Rasik, A.M., Jain, G.K. and Shankar, R. 1998. *In Vitro* and *in Vivo* wound healing activity of asiaticoside isolated from *centella asiatica*. *Journal of Ethno pharmacology* 65 (1999): 1-11.
- Shukla, R. and Jain, A.P. 2018. Wound healing effect of ethanol extract from morning Glory, *Ipomoea Carnea Jacq.* leaves by using different models in rats. *Pak. J. Pharm. Sci.*, 31, No. 4.
- Singer, A.J. and Clark, R.A.F. "Cutaneous wound healing," *The New England Journal of Medicine*, 341(10):738–746, 1999.
- Singh, M.P. and Sharma, C.S. 2009. wound healing activity of *Terminalia Chebula* in experimentally induced diabetic rats. *Int. J. PharmTech Res.*; 1: 1267-1270.
- Steenkampa, V., Mathivhaa, V., Gouwsb, C.E.J. and Rensburga, D. 2004. Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa, *Journal of Ethnopharmacology* 95, 353–357.
- Stewart, R. 1997. "Herbalism: most common form of medicine available," *The Eastern Pharmacist*, 475.
- Suntar, I.P., Koca, U., Esra, K.A., Yilmazer, D. and Alper, M. 2009. Assessment of wound healing activity of the aqueous extracts of *Colutea cilicica* Boiss. Bal. fruits and leaves.

- Suruse, P., Kale, M.K., Gunde, M., Amnerkar, N. and Pathak, A.K.2011. Evaluation of wound healing activity of *Arisaema leschenaultii blume*. In rats. *Pharmacia letter*, 3: 200-6.
- Tatiya, A.U. and Hatapakki, B.C.2003. "Anti-inflammatory activity of bark of *Machilus macrantha* Nees. *Lauraceae*", *IJPS*, 532- 34.
- Tatiya, A.U. and Saluja, A.K.2011. "Further studies on membrane stabilizing, anti-inflammatory and FCA induced arthritic activity of various fractions of bark of *Machilus macrantha* in rats, *Rev bras Farmacogn*,21 (6): 1052-1064.
- The wealth of India, raw material. "council of scientific and industrial research" 2005.
- Tsuchiya, H. and Sato, M., *et al.*,1996. "Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*," *Journal of Ethnopharmacology*,50 (1): 27-34.
- Udapa, A.L., Rao, S.G. and Kulkarni, D.R. 1989. Wound healing profile of septilin. *Indian Journal of Physiology and Pharmacology*, (33): 39 - 42.
- USDA NRCS Nacogdoches.2001. *TX Technical office and the National plant data center*, INDIA HELIOTROPE *Heliotropium indicum* Michx.
- Verma, V.K., Boruah, M.P., Bais, M. and Nandy, B.C. 2015. Wound healing activity of the ethanolic extract of *Solanum spirale* leaves indigenous to north east India, *Journal of Drug Discovery and Therapeutics*, 3.
- Vijay, L. and Kumar, V.2012. Effect of *Moringa oleifera* Lam. on normal and dexamethasone suppressed wound healing. *Asian Pacific Journal of Tropical Biomedicine* 2: 219-S223.
- Vinothapooshan, G. and Sundar, K. 2010, wound healing effect of various extracts of *Adhatoda vasica* ISSN 0975 – 6299, 1.

- Ya, C, Gaffney, S.H., *et al.*, 1988. *Chemistry and significance of condensed Tannins*, Plenum Press, New York, NY, USA.
- Yasukawa, K., Akihisa, T. and Yoshida, Z.Y. 2002 Inhibitory effect of euphol, a triterpene alcohol from the roots of *Euphorbia kansui*, on tumor promotion by 12-o-tetradecanoylphorbol- 13-acetate in two-stage carcinogenesis in mouse skin. *J Pharm Pharmacol*, 54: 119-124.
- Yeo, D. Attioua, B., Lehalle, C., Kossi, M., guessan, J.D., Djaman, A.J., Lobstein, A. and Frossard, S. 2011. Isolation of wound healing compounds from *Heliotropium indicum*. *Journal of Applied Pharmaceutical Science* 01 (10): 102-106.
- Zahra, A., Kadir, F.A., Mahmood, A.A., Al hadi, Suzy, S.M., Sabri, S.N., Latif II and Ketuly K.A.2011, Acute toxicity study and wound healing potential of *Gynura procumbens* leaf extract in rats. *Journal of Medicinal Plants Research*; 5: 2551-58.

VIII APPENDICES



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

Dr.P.R.Padma

Dean, School of Biosciences

Professor and Head

Department of Biochemistry, Biotechnology and Bioinformatics

CERTIFICATE

This is to certify that the project title "*In vivo* wound healing activity of *Heliotropium indicum* Linn. leaves" has been approved by the IAEC.

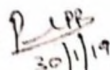
Name of the chairman, IAEC

Dr.P.R Padma

Name of the CPCSEA Nominee

Dr. C. Gunasekaran

Signature with date

Chairman, IAEC: 
30/1/19

CPCSEA Nominee: 
30/1/19

