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Phytochemical Investigation and Biological Activity of Leaves Extract of Plant BoswelliaSerrata

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Before onset of synthetic era, man was completely dependent on medicinal plants for prevention and treatment of diseases. Medicinal plants, herbs, spices and their remedies are known to ayurveda in India since long times. The value of medicinal plants, herbs and spices as herbal remedies is being lost due to lack of awareness and deforestation. Hence as a result, many valuable medicinal herbs are becoming rare and thus the precious information is lost[35].Of the estimated 500,000 plants on our planet, it is thought that around 10,000 are regularly used for medicinal purposes. Significant proportions of these herbs have been well researched and the most are excellent for home use. The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in history of all civilizations. The Indian system of herbal medicine has been regarded by many of the western scholars. It has been said that Indian herbal medicine is a rich mine of knowledge from which many useful things might be unearthed. The earliest mention of the medicinal use of plants is to be found in the Rig-Veda, which is one of the oldest repositories of human knowledge, having written between 4500 and 1600 BC.

Even in late as the 1930s, around 90% of medicines prescribed by doctors are sold over the counters were herbal medicines. The major pharmaceutical companies now a days have realized that rainforest; grasslands and even had growers and fields are sources of potentially invaluable medicines. As a result they are investing large sums of money to try to find new plant chemicals that can be marketed as medicines.

*Keyword:*Leaves of Boswelliaserrata, Flowers of Boswelliaserrata, Gum of Boswelliaserrata, Bersuracea.

1. Introduction

Nature always stands as a golden mark to exemplify outstanding phenomenon of symbiosis. Nature has provided a complete store house of remedies to cure ailments of mankind. The knowledge of drug has accumulated over thousands of year as a result of man's inquisitive nature. The retrieval of knowledge on the use of medicinal plants has served as a valuable tool in the discovery of new drugs. Plants serve various purposes in the world. Their usefulness can be in the form of food, shelter, religious, medicines etc. Over 4, 00,000 species of tropical flowering plants have medicinal properties.

In recent years, natural product especially those derived from plant sources is gaining much interest for therapeutic use than that of the conventional ones. This is due to development of resistance and unwanted side effects and other problems resulting from the use of synthetic

drugs[1]. But product from natural source exhibit minimal resistance and negligible side effects though they are well tolerated.

The history of herbal medicine is as old as human civilisation. Since old times before modern medicine, people became ill and suffered from various ailments. In absent of modern medicinal remedies people relieved on herbal remedies derived from herbs and spices. There are many medicinal herbs and spices, which find place in day-to-day uses, many of these, are used as herbal remedies. Many cooked foods contain spices. Some minor ailments like common cold, cough, etc. may be cured by herbal remedies with use of medicinal properties of spices. Herbal remedies can be taken in many forms. Infusions are steeping herbs or spices, with parts like leaves and flowers with boiling water for some time. Filtered or unfiltered use this water extracts of spices as herbal remedies. Decoction from boiling roots, bark other parts of herbs and spices with water for a long time. Infusion and decoction both are known from herbal teas. Sometimes essential oil of herbs and spices are also used as herbal remedies[2].

The alternative system of medicine like ayurveda, siddha, unani and other tribal folklore medicines have significantly contributed to the health care of population of India. Today, these systems are not only complementary but also competitive in the treatment of various diseases. Initially the materials employed in these traditional medicines were almost botanical origin.

Ayurveda is a holistic health science, having diversity, flexibility, accessibility, affordability and have a potential to meet the new challenge to human life. As an alternative form of medicine, unani has found favour in Asia which is very close to ayurveda. Ayurveda (Davanagari: the 'science of life') is a system of traditional medicine native to the Indian subcontinent and practiced in the other parts of the world as a form of alternative medicine. According to the followers of Unani medicine, the five elements are present in different fluids and their balance leads to health and their imbalance leads to illness.

The universal role of medicinal plants in the treatment of disease is exemplified by their employment in all the major systems of medicine, irrespective of the underlying philosophical premises. The survey of literature of Indian system of medicine reveals that medicinal plants were in use as early as 4500 B.C. Nature has bestowed on us a very rich flora and fauna in addition to very diverse marine and microbial resources of natural product. Due to wide variation in climate, soil, altitude and latitude, India has medicinal flora and is largest procedure of medicinal herbs next to china. It has the reputation of being one of the world's leading biodiversity centers due to the presence of over 45,000 different plant species, India has 16 different agro climatic zones, 10 vegetation zones, 25 biotech provinces and 426 biomes, 1,800 species of flowering plants[3].

The use of medicinal plants for both primitive and curative therapies is not new as records of indigenous knowledge from various plant of the world illustrate an age long tradition of plant being a more bio resource base for health care. The documents many of which are of great antiquity revealed that the plants used for medicinal purposes were notified in India and other countries too. In the nineteenth century, the plant have played a major role as the basic source for the establishment of several pharmaceutical industries which are important for establishing and enhancing the economy of a developing country like India. Over 75% of the world population relies mainly on plants and plant extract for health care and more than 30% of the entire plant species, at one time or other, were used for medicinal purpose

The Word Health Organization (WHO) encourages the inclusion of herbal remedies that have been proven to be efficacious and safe, into primary health care. The long historical use of medicinal plants in many traditional medicinal practices, including experience passed from generation has demonstrated the safety and efficacy of traditional medicine. However, scientific evaluation is needed to provide evidences of their safety and efficacy (WHO, 2000). Several chemotherapeutic agents have

been developed in the modern system of medicines as a result of screening of medicine of the medicinal plants in various parts of the world. The isolation of biologically active phytoconstituents such as alkaloids, Quinine, serpentine, reserpine, norcotine, caffeine, nicotine, etc, are result the initial leads obtained from the traditional system of medicine. Explanation of chemical constituents of plants and pharmacological screening may provide us the basis for the developing the leads for the development of novel agents for curative purposes for treating various illness like diabetes, cancer, sexually transmitted diseases neurological and immunological disorders etc.

Plants contain number of metabolites. Among the estimated 250,000-500,000 plants species only a small percentage has been investigated phytochemically. Solvent fractions of them have been submitted for biological screening. The process of evaluation of plants for various pharmacological activities is a much time consuming/requiring large number of man hours. Natural products have proven to be the richest source of medicinal compounds. Although, many drugs are prepared by synthesis, most of the core structures of scaffolds for synthetic chemical are based up on natural products[4]. The key advantage of natural products over synthetic compounds is their chemical diversity. Almost one half of the chemical scaffolds from natural products cannot be reproduced by synthetic chemistry. Novel natural products and proprietary chemical scaffolds are in high demand by the pharmaceutical and agrochemical industries because compounds with novel chemical entities can be used to generate chemical libraries by parallel synthesis[5].

Phytochemistry is the art of resolving plants into chemically pure individual constituents. As a result of recent interest in the plant kingdom as a potential source of new drug strategies for the fraction of plant extracts based on biological activity rather than a particular class of compound developed. The chemical examination follows after the isolation and biological screening of the active fraction[1].

The structure determination and biological activity screening of natural products, especially those with a history of medicinal use taking clues from folklore medicines, ayurveda ,tribal medicine etc., has been an important activity in medicinal chemistry. The plants often synthesise unexpected and novel structures by biosynthetic path to protect themselves from predator organisms. In spite of the fact, at present, we have at our command a formidable array of modern drugs andthe need to discover and invent new agent is genuine and primordial[6]. It has been estimatedsatisfactory therapy is available only for about one third of all presently known human ailments, and several diseases such as cancer, AIDS, senile dementia, autoimmune diseases[7].

Approximately one third of the prescription drugs in the United States contain plants[8] and more than 120 important prescription drugs derived from plants[9]. Most of these drugs were developed because of their use in traditional medicine. Recently WHO studies indicate that over 30% of the world's plant species have at one time or another been used for medicinal purposes of the 2,50,000 higher plant species on earth, more than 80,000 species are medicinal. Although traditional medicine is wide spread throughout the world, it is an integral part of each individual culture. Its practise is based mainly on traditional belief handed down from generation for hundred or even thousands of years.

The Chinese were the first to take full advantage of medicinal plants. Over 5000 years ago, the emperor ShenNung studied medicinal plant and verified the pharmaceutical properties[10]. Over 11000 herbal remedies were developed and used in china for thousands of years. These include Ma Huang, also known as ephedra, the sources of the classic adrenergic drug ephedrine and artemisia. The source of artemisinin, a new antimalerial drug is currently under joint development by the WHO and the US for a long time, the only way to use plant medicine was either direct application or the use of crude plant extracts. Now, it is possible to rapidly build up extensively, libraries of certain classes of organic compounds by the method of combinatorial chemistry[11,12].

The structural determination and synthesis of natural products has received attention only during the early 19th century and extensive investigation of medicinally useful natural product is still in progress. A natural product lead structure is subjected to chemical modification to arrive at the therapeutically important molecular fragments, the pharmacophore only a few natural products are directly used as drug but in many cases the chemical modification of the lead structure gave a more potent synthetic and semi synthetic analogue[13].

A viable program of drug development, aimed at providing cheap drugs in this country, must take the following factors in to consideration. i) The prevailing socio-economic condition and the high cost of medical care. ii) Our vast and over increasing population, a majority of them live in rural areas often far away from means of communication.

Human beings are in the race of endemic and epidemic diseases since ages and have been a potential hunter in search of medicines from the nature. Illness has been a serious concern with the pain and suffering by the patients affected from various bacterial, viral, fungal, protozoa and parasitic diseases. Some of the diseases like typhoid, malaria, tuberculosis, ulcers, cancer, AIDS, syphilis, leprosy, diabetes etc are being treated with herbal therapies.

Ulcer is a serious gastrointestinal disorder that requires a well-targeted therapeutic strategy. A number of drugs including proton pump inhibitors and H_2 receptors antagonists are available for the treatment of peptic ulcer, but clinical evaluation of these drugs has shown incidence of relapse, side effects, and drug interactions. This has been the rational for the development of new antiulcer drugs and the search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse.

Amongst the various diseases, the parasitic infection is one of the major challenges for the health care industries. Helminthes are recognized as a major problem to livestock production throughout the tropics. Parasitic helminthes affect human being and animals by causing considerable hardship and stunted growth. Most diseases caused by helminthes are of a chronic and debilitating in nature. The parasitic gastroenteritis is caused by mixed infection with several species of stomach and intestinal worms, which results weaknesses, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity. Although some synthetic drugs are available to control such kind of infections but due to their high cost and untoward effects, the development of more effective and safe drugs from reasonably less expensive natural sources is our main consideration. This can rationally be approached through the study of indigenous traditional plant remedies[14].

Anthelmintics are therapeutic agents used to destroy parasitic worms or remove them from the infected host. The majority of helminth infections are acquired by contact with (a) Infected animals (b) Ground contaminated by human or animal excrement (c) Water infected with cerearіae, and (d) Indestion of a infected meat. The filarial worms require arthopod vectors, such as blood sucking mosquitoes, which transmit the parasite from one host to another.

Surveys have shown that one-third of the human race suffers from helminth disease, of which a large number are multiple infections, though helmintic infections are usually associated with tropical regions. More than 40 million Americans are also victims of this infection.

Table1: Drugs obtained from herbs and plants, source, therapeutic activity and their synthetic or semi-synthetic analogs.

Sl. No	Drugs from herbs and plants	Source	Therapeutic activity	Synthetic or synthetic analogs
1	Atropine	*Atropa belladonna*	Antimuscarinics	DicyclomineHCl, Hyoscinebutylbromide
2	Benzyle penicillin	*Penicillin chrysogenum*	Antibiotic	Ampicillin, amoxicillin.
3	Codeine	*Papaversomniferum*	Analgesic	Nolorphine, mepiridine.
4	Camptothecine	*Camptothecaaccumnata*	Anticancer	10-hydroxycaptothecine, aminocamptothecine, topotecan,

				ironotecan.
5	Digoxin	*Digitalis lanata*	Cardiovascular	-
6	Ephedrine	*Ephedra vulgaris*	Anti-asthma	Salbutamol, salmeterol.
7	Lovastatin	*Aspargillusterrus*	hypercholesterolemia	Pravastatin.
8	Morphine	*Papaversomniferum*	Analgesic	Heroine, naloxane, phthadine.
9	Podophyllotoxine	*Podophillumpettatum*	Anticancer	Etopside, toniposide.
10	Quinine	*Cinchona succirubra*	Antimalerial	Chloroquine, meploquinine, pamaquine, premquine.
11	Reserpine	*Rawolfiaserpentina*	Hypotension and anticholinergic	-
12	Tubacurarine	*Tube curare*	Neuromuscular blocking agent	Decamethoxium, soxamethorium.
13	Taxol	*Taxusbaccata*	Anticancer	-
14	Teprotide	*Bathrops*	Antihypertensive	Captopril, enalapril, lisinopril.
15	Vinblastin, vincristine	*Cathranthusroseus*	Anticancer	Vindesine.

Inaddition, these diseases present a serious economic problem to the animal is vulnerable to a large number of parasite worm infections. Next to schistosomiasis, hookworm disease and ascariasis are not most prevalent serious human infection in animals. The most serious helminthiasis is caused by flukes and round worm. Table1. Presents some of the clinically very important natural product drugs, scaffold structures, synthetic or semi-synthetic analogs.

Globally, there has been an unparalleled growth in the plant-derived medicinally useful formulations, drugs and health-care products. Its market covering more than 60% products derived from plant origin. India exhibits remarkable outlook in modern medicines that are based on natural product besides traditional system of Indian medicines. Almost, 70% modern medicines in India are derived from natural products. Medicinal plant plays a central role not only as traditional medicines but also as trade commodities, meeting the demand of distant markets. Ironically, india has a very small (1.6%) of this ever-growing global market. To compete with the growing market, there is urgency to expeditiously utilise and scientifically validate more medicinally useful plants while conserving these species, which seems a difficult task ahead[15].

1.1 Medicinal and Aromatic Plants

India has 2.4% of world's area with 8% of global bio-diversity. It is one of the 12 mega-diversity hot regions of the world. Other countries being Brazil, Colombia, China, South Africa, Mexico, Venezuela, Indonesia, Ecuador, Peru, USA and Bolivia. Across the country, the forest of India is estimated to harbour 90% of India's medicinal plants diversity in the wide range of forest type that occur. Only about 10% of the known medicinal plants of India are restricted to non-forest habitats. The estimated numbers of plant species and those used for medicinal purpose vary. According to one fifth of all the plants found in India are used for medicinal purpose. The world average stand at 12.5% while India has 20% plant species of medicinal value[16] But according to Hamilton (2003), India has about 44% of flora, which is used medicinally. Although it is difficult to estimate the number pf medicinal and aromatic plants present worldwide, the fact remains true that India with rich biodiversity ranks first in percent flora, contains active medicinal ingradient[17].

Table 2: (a) Number of plants used medicinally worldwide.

Country	Plant species	Medicinal plant species	Percentage
China	26,092	4,942	18.9
India	15,000	3,000	20.0
Indonesia	22,500	1000	4.4
Malaysia	15,500	1,200	7.7
Nepal	6,973	700	10.0
Pakistan	4,950	300	6.1
Philippines	8,931	850	9.5
Srilanka	3,314	550	16.6
Thialand	11,625	1,800	15.5
USA	21,641	2,564	11.8
Vietnam	10,500	1,800	17.1
Average	13,366	1,700	12.5
World	422,000	52,885	-

Table 2: (b) number and percentage of medicinal plant species recorded for different countries and regions[17].

Country or region	Total no. of native species of flora	No. of species of medicinal plants	% of flora which is medicinal
China	27,100	11,146	41
India	17,000	7,555	44
Mexico	30,000	2,237	7
North America	20,000	2,572	13
World	297,000-510,000	52,896	10-18

The existence of traditional depends on plant species diversity and the related knowledge of their use as herbal medicine. In addition both plant species and traditional knowledge are important to the herbal medicine trade the pharmaceutical industry whereby plants provide raw materials and the traditional knowledge prerequisite information[18].

India has the richest plant medical tradition in the world. It is a tradition that is of remarkable contemporary relevance for ensuring health security to the teeming millions. There are estimated to be around 25,000 effective plant-based formulations, used in folk medicine and known to rural communities in India. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications. It is estimated that there are over 7800 medicinal drug-manufacturing units in India, which consume about 2000 tonnes of herbs annually[19]. The market for ayurvedic medicine is estimate to be expanding at 20% annually. Sales of medicinal plants have grown by nearly 25% in India in past ten years (1987-96), the highest rate of growth in the world[20]. Two of the largest users of medicinal plants are China and India (figure.1). Traditional Chienies Medicine (TCM) use over 5000 plant species; India uses about 7000[21, 22].

What is an herbal medicine?

Herbs has various meaning, but in the context of this article it refers to "crude drugs of vegetable origin utilized for the treatment of diseases states, often of a chronic nature, or to attain or maintain a condition of improved health". Herbal medicine sometimes referred to as Herbalism or Botonical medicine is the use of herbs for their therapeutic or medicinal value. An herb is plant or plan part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substance that act upon the body. Herbal preparations are called "Phytopharmaceuticals", "Phytomedicinal" or "Phytomedicine", are preparations made from different parts of herbs or plants. They come into different formulations and dosage forms including tablets, capsules, elixir, powder, extract, tincture, cream and parentral preparations. A single isolate or active principle derived from plants such as digoxin or reserpine tablets is not considered herbal medicine[23, 24].

Why herbal remedies?

Their effectiveness, easy availability, low cost and comparatively being devoid of serum toxic effects popularised herbal remedies.

Popularity of herbal medicine

Theherbal medicine is largely gaining popularity over allopathic medicine because of the following reasons favorable to it.

1. Rising costs of medicinal care.
2. As these are from natural origin, so free from side effects in several cases.
3. Goes to root cause and removes it, so that the disease does not occur again.
4. Freedom from approaching various specialists.
5. Cure for many obstinate diseases.
6. Easy availability of drugs from natural sources.

1.2 Need and scope of herbal therapy

The treatment of diseases with pure pharmaceutical agent is a relatively modern phenomenon. However, as European explores and merchants' spreads out to the Western and Eastern parts of the world, some of the benefits

they would bring back were discovered pharmaceutical preparation of natural origin. One of the earliest success stories in developing a drug from a natural product was aspirin.

Today we are more concentrated with life-style disease like depression, cancer and heart troubles caused by faulty nutrition and stress. The need of alternatives therapy is to cover a good health for all. Herbal therapy is one of the best practices to overcome the illness.

Traditional Indian practice held that certain drugs should be formulated through the addition of chosen substance that enhances bioavailability enhancer property and point to the active component as the molecule piperine.

An anti-TB drug Rimfampicin has to be given at higher dose that required in order to compensate for losses on the way to the target site. Formulation of Piperine with Rifampicin will have the counter effects[25,26].

1.3 Herbal side effects

Little is known about Phytomedicine safety. There has been increased in the number of side effects reported in the literature[27]. Many case however, could have gone unreported because herbal medicine usually self-prescribed and often times ignored by health practitioners during the patients care. Identifying adverse effect is further hindered because it is not always possible to assess the quality of certain herbal medicinal products.

1.4 Herbal medicine drug-interactions

The potential risk of an herbal medicine interacting with a prescribed drug is also a concern with the increased use of Phytomedicine. Recently, several interactions have drawn the attention of the medical community. Janetzky and Morrealc reported a probable interaction between *ginseng* one of the most popular herbs with multiple health claims and warfarin, drug with numerous well-recognised drug-drug interaction.

Understanding Drug-Herb Interactions

Drug interactions occur by 4 major mechanisms.

1. Altered drug absorption
2. Altered renal (kidney) elimination of drugs
3. Additives effect or toxicities (Pharmacodynamic interaction).
4. Altered hepatic (liver) metabolism of drugs.

The first three account for a relatively small number of problems, while the fourth is the major culprit in drug interactions. The potential seriousness of a drug interaction depends in part on which drugs are involved. Some drugs have what is called a “narrow therapeutic margin” which means that there is a relatively small difference between the amount of drug needed to achieve its beneficial effect and that causing adverse or unwanted effects. Classical examples of drug falling in this category are anticoagulant (blood thinners like warfarin), which can cause bleeding if the relative amount of drug is increased as a result of a drug interaction, anticonvulsants (anti-seizure medications such as phenytoin) and the heart drug digoxin.

1. Absorption

When drugs are given orally they are usually absorbed into the bloodstream through the stomach. Or by drug absorption may be due to alteration in pH, or acidity of the stomach, or by drugs binding together in the stomach to form complexes which cannot then be absorbed, for example because the molecule is too large to pass through the intestinal wall. Common examples include antacid, which increase stomach pH, and iron supplement, which can bind to some antibiotics, such as ciprofloxacin or tetracycline.

Another issue for absorption is the “motility of the gastrointestinal tract”, in other words, how fast or slow your guts are moving. If you have diarrhea, the drugs or herbs are moving through your system quickly and may have less time to be absorbed. Laxative or bulk-forming agents speed up intestinal transit, and might interfere with intestinal absorbed drugs. Common stimulant laxative herbs are anthranoid-containing plants like *senna, frangula, yellow dock* and Chinese *rhubarb*, as well as *Cascara sagrada* and *Aloe-vera* leaf. Bulk-forming agents include guar gum

and *psyllium*. The clinical significance of these interactions is not clear.

2. Elimination

Drug interaction due to alteration in elimination of drugs through the kidney can only occur if a drug is primarily eliminated from the body through the kidney. If a drug or herb causes decreased kidney function, levels of the drugs eliminated through the kidney may be increased as a result. Herbs containing diuretic properties, such as corn corn silk, dandelion, and juniper can increase the toxicity of lithium, a drug used to treat bipolar disorder.

3. Pharmacodynamic interactions

Some drugs (and herbs) that may be given together have similar beneficial effects, or similar toxic effects this is called a Pharmacodynamic interaction. For example, two antiretroviral drugs may both cause the side effects of peripheral neuropathy, increasing the likelihood of that side effect developing. Many drug-herb interactions fall in this category. For example herbs that have sedative properties, such as kava, nettle and sage may increase the sedative effect of some sleeping medications. Herbs that have antiplatelate activity, such as ginkgo biloba, ginger, ginseng and garlic may increase the risk of bleeding in patient taking traditional drugs with antiplatelet activity or blood thinners. Herbs that can increase blood pressure, such as blue *cohosh, ginger, liquorice* and bayberry can interfere with the effectiveness of drugs used to treat high blood pressure.

4. Liver Metabolism

The most complicated drug interactions, and those with greatest significance for antiretroviral medication, are those resulting in altered liver metabolism of drugs. The activity of liver enzymes which are responsible for breaking down drugs can be increased (induced) or decreased (inhibited) by drugs or herbs. Many antiretroviral medications are enzyme inducers, enzyme inhibitors, or even both at the same time. The resulting drug interactions are complex, and not always predictable.

Ritonavir, a protease inhibitor, is a powerful inhibitor of liver metabolising enzymes, and can dramatically increase the blood level of the other drugs metabolized by the same enzymes. This interaction can be used to our benefit, so that lower doses of the drugs affected are required to achieve the same effect. If dose adjustments are not made however, toxic levels of the affected drug could result. Nevirapine, another antiretroviral is an enzyme inducer, and can decrease the blood levels of other drugs metabolized by the same enzyme. If we know how each drug is metabolised and how it affects metabolizing enzymes, we can predict the response and be prepared.

Many drugs in a wide variety of therapeutic categories are metabolized by liver enzymes and subject to this type of interaction. These include drugs used to treat anxiety and insomnia (diazepam and some of its relatives), drug used to treat depression, some anti-arrythymics (used to treat abnormal heart rhythms), oral contraceptives, painkillers and recreational drugs.

1.5 Common medicinal plants reported to interact with pharmaceuticals

A recently published comprehensive search of interactions between commonly used medicinal plants and pharmaceutical drugs published in clinical reports suggest potential interactions with the following herbals, betel nut, chilli pepper(capsicum), Danshen, Devils claw, dong quai, eleuthero or Siberian ginseng, garlic, gingkom guar gum, karela or bitter melon, liquorice, papaya, psyllium (St. John's wort, Saiboku-to Asian herbal mixture); Shankhapushpi (Ayurvedic mixed –herb syrup); Sho-saiko-to or xiao chai hu tang (Asian herbal mixture), tamarind, valerian and yohimbine.

Some specific cautions are that people with clotting disorders, those awaiting surgery, or those on anticoagulant therapy should be aware that ginkgo, Danshen, dong quai, papaya, garlic, feverfew, ephedra or ginseng may cause unexpected bleeding, increase bleeding times or inhibit blood clotting for about two week after you stop taking the herb. People taking protease inhibitors, serotonin is uptake inhibitors (newer

antidepressants), cyclosporine, digoxin, phenprocoumon need to consider potential interactions with St. John's wort. Ginseng may interact with phenelzine, another antidepressant. People taking tricyclic antidepressant should avoid yohimbine. Liquorice, which has been shown to have antiviral properties and is a very common ingredients in Chinese herbal remedies, can have an additive synergistic effect with corticosteroids. Corticosteroids, like prednisone are commonly prescribed to ulcers in the throat and mouth that don't respond to topical preparations, or to treat rashes associated with some antiretroviral. These are only some of the herb-drug interactions for which clinical reports have been made. Many others are possible, and indeed likely. The complexity and potential gravity of drug herb interactions makes exercising caution and consulting a pharmacist or physician important[28].

1.6Toxicology and Herbs

In recent years there have been reports of death and poisonings attributed to the use of medicinal plants such as comfrey and chaparral. Herbalists have a responsibility to determine the inaccuracies in such reports. Literature concerning poisonous plant is replete with misinformation and erroneous reporting[29]. These same mistakes continue to plague the reporting of poisoning by medicinal plants[30, 31].

What is toxicology?

The word toxicology is derived from *toxicon*-a poisonous substance into which arrow heads were dipped and *toxikos*-a bow. Toxicology is relative young biological science that involves a complex interrelationship among dose, absorption, distribution, metabolism and elimination.

What is a poison?

A poison is substance which has a harmful effect on a living system. Paracelsus (1493-1541) was one of the first to distinguish between the therapeutic and toxic properties of substances.

He thought the only difference between a medicine and a poison was the dose. Very few substances are actually classed as a "poison". Harmful chemicals are not necessarily poisons. We are exposed to potentially toxic substances every day without immediate harm. Our bodies can usually safely metabolize toxins if we are exposed to them in small amounts. It is only when we overwhelm our body and reach the toxic dose of a substance that life threatening results occur. That is all substances have a potential toxicity. All herbs can therefore be harmful, but most would have to be ingested in impossible amounts to cause harm. Herbs which have a high toxicity, such as Gelsemium and Aconitum, can be used safely and effective if taken in a small, therapeutic dose. Thus, the primarily determinate of the safety of a substance is the dose, not the herb, which makes the poison. A correct use of semantics and a correct understanding of these terms are crucial to avoid confusion and misinformation.

For example

- Vitamin D has a very high acute toxicity. It would have to carry a poison label but it has been exempted from the federal Hazardous substances labelling act because it is classed as a food and a drug.
- Salt is not toxic in small doses. But a single large dose can be lethal. Just two tablespoon can kill a one-year-old child.
- Caffeine one of the many alkaloids found in coffee can kill-at a dose of 100 strong cups of coffee.
- A litre of scotch contains a lethal dose of ethanol.
- Water can be lethal if drink enough of it in a short period of time.

Toxicology sometimes refers to potentially poisonous substances as xenobiotics after the Greek *xenos*, strange, and bios, life. These are substances that are foreign to the body or exogenous as compared to substances produced by the body or endogenous. It is important to point out that endogenous substances can also cause poisoning and death.

Toxic substances fall into several classes in relation to how people are exposed to them. They can be classed as food additives, drugs, pesticides, industrial chemicals, environmental

pollutants, household poisons and natural toxins, many natural products used in medicine are derived not only from plants, but also from marine organism like starfishes, sea urchins, sea cucumbers and fish. Chinese medicine makes use of chemicals produced from animal such as antelope horn, worms, scorpions and bee hives. Every substance has potential toxicity, from the most benign to the most obvious. Even so, the same dose will not affect every person in the same way. It is this fact which makes toxicology such a complex science. It is not enough to say a substance is toxic. There are a myriad of factors which may make the substance more or less toxic to particular individual.

1.7 Dose response relationship

Toxicity depends not only on the dose of the substance but also on the toxic properties of the substance. The relationship between these two factors is important in the assessment of therapeutic dosage in pharmacology and herbalism[32].

1.8How plant substances can harm?

Toxicants can interrupt metabolism of carbohydrates, lipids and proteins and alter synthesis, release and harmones. Here are some examples of how substance from plant can harm; Oxalate crystals from *Halogetonglomeratus* can damage the tubules in the kidney because they insoluble precipitate and collect in the kidney tubules which then obstruct them. The alkaloid, aconitine in aconite, affects the sodium channels on the cell membrane which can lead to increased uptake in sodium and other ions. This can lead to cardiac arrhythmias and depression of respiration.

Tubocurarine, the most potent constituent in curare denies access to neurotransmitters in nerve receptors. This results in paralysis of the muscles, including the respiratory muscles. The psychotropic plant alkaloids, harmine and harmaline resemble serotonin and are thought to block the serotonin receptors in the brain. Morphine and other opiates bind to neurotransmitters receptors in the brain. In large amounts they can depress the respiratory centre in the brain. Glucosinolates are compound found commonly in plant of the mustard family. Some can be powerful irritant to eyes, skin and the respiratory tract. Some alkaloids are distrupt nerve tissue such as coniine from poison hemlock (*conium*) causing nervousness, trembling, bradycardia and fatal paralysis. Saponins can gastric upset because of its "soapy" properties which interfere with digestion[27].

India is a varietals emporium of medicinal plants. It is one of the richest countries in the world as regards genetic resources of medicinal plants. Even though the land mass of India occupies only 2% of the globe, it occupies 11% of the total known world flora and is one of the world's top 12 mega diversity nations. Two of the '18 hot spots' in the world are in India. The Indian system of medicine, ayurveda Siddha and unani uses over 2000 medicinal plants of which ayurvedic system of medicines uses about 700, sidda-600 and unani-700 medicinal plants in our country[33]. Plants and other natural substances have been used as the rich source of medicine. All ancient civilizations have documented medicinal uses of plant in their own ethnobotanical texts. The list of drugs obtained from plant source is fairly extensive[34].

2. Materials and Methods:

Chemicals:

Petroleum ether (40^0- 60^0), benzene, chloroform, ethanol, methanol, silica gel-C (60-120 mesh) and silica gel-G (S.D. Fine Chem. Ltd. Mumbai), Tween-80, Piperazine citrate (Glaxo Smith line Ltd.), Lansoprazole (Lee Pharmaceuticals, Hyderabad)Aspirin **(**Shalg pharmaceuticals, Goregaon, Mumbai**)** and all other chemicals used were of the analytical grade.

2.1 Collection of Plant Material:

*Boswelliaserrata*were collected from the Western Ghat, Satara district, Maharashtra (India) in the flowering month of June-July 2010. The plant material was identified and authenticated by Dr.P.G.Diwakar, Botanical survey of India, Pune (KP/09/1203).

2.2 Helminth:

Pheretimaposthuma(Indian adult earthworms) of 3-5 cm in length and 0.1-0.2 cm in width were procured from the Agriculture Research Centre, Gulbarga (Karnataka). All the earth worms were pre-treated with normal saline solution to remove the complete faecal matter.

2.3 Animals:

Albino wistar rats of either sex weighing between 150 to 200 gm and Albino mice of either sex weighing between 20 to 25 gms. Were procured form registered breeders (149/1999/CPCSEA, Mahavir Enterprises, Hyderabad). The animals were housed under standard conditions of temperature (25 ± 2^0C) and relative humidity (30-70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (VRK Nutrition, Pune) and water *ad libitum*. Approval at the Institutional Animal Ethics Committee (IAEC) of R.M.E.S's College of pharmacy, Gulbarga was taken for conducting antiulcer activity.

2.3Extraction of plant material:

The shade dried leaves of *Boswelliaserrata* was powdered to 22 mesh size and then subjected to successive soxhlet extraction using highly non-polar to polar solvent systems such as petroleum ether (40-60^0 C), chloroform, ethanol and distilled water until the solvent became colourless. The extracts obtained were further evaporated to dryness under vacuum and stored in the refrigerator for further use.

2.4 Preliminary phytochemical screening:

Preliminary phytochemical screening is carriedout to determine the presence of various bioactive constituents in the crude extracts of petroleum ether (EE), chloroform (CE), ethanol (AE) and distilled water (AE) [84, 85, 86].

2.5 Tests for Carbohydrates:

1. **Molisch's test**: To 2-3 ml of extract, 1-2 drops of α-naphthol solution in alcohol is added, shaken and then concentrated H_2SO_4 is added along the walls of the test tube, a violet ring appears at the junction of two liquids.
2. **Iodine test**: To the test solution, 2-3 drops of iodine is added and then observed for blue colour occurrence.
3. **Fehling's test**:Equal volume of Fehling's A and Fehling's B reagents are mixed and few drops of test solution is added in a test tube and heated in boiling water bath for 5-10 min and observed for a yellow, then brick red precipitate appears.
4. **Benedict's test**:To 2-3ml Benedict's reagent and few drops of the test solution is mixed thoroughly in a test tube and then heated in a boiling water bath for 10-15 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in test solution.
5. **Barfoed's test**: Equal volume of Barfoed's reagent and test solution added in a test tube is heated for 1-2 min in boiling water bath and then cooled. Observe for red granular precipitate is observed at the bottom of the test tube.
6. **Selwinoff's test**:HCl reacts with ketoses to from derivatives of furfuraldehyde which gives red coloured compound when linked with resorcinol. Add compound solution to about 5ml of reagent and boil. Fructose use red color within half minute. The test is sensitive to 5.5mmole/lit, if glucose is absent but if glucose is present it is less sensitive and in addition large amount of glucose can give a similar colour.

Tests for Proteins:

1. **Biuret test**: To 3 ml of the test sample, 4% NaOH and few drops of 1% $CUSO_4$ solution is add. Violet or pink colour is formed.
2. **Ninhydrin test**: Amino acid and proteins when boiled with 0.2% solution of ninhydrin (indane-1, 2, 3-trionehydrate) violet colour appears.
3. **Millon's test**: Mix 3 ml T.S. with 5 ml Million's reagent, white precipitate obtain. Precipitate warm turns brick red or precipitate dissolves giving red colour.
4. **Xanthoprotein test**: 3ml of test sample is mixed with 1 ml of concentrated H_2SO_4, white precipitate is formed.
5. **Test for protein containing sulphur**:Mix 5 ml T.S. with 2 ml 40% NaOH and 2 drops 10%

lead acetate solution. Solution was boil, turns black or brownish due to $PbSo_4$ formation.

6. **Precipitation test**:The test solution was observed for white colloidal precipitate with following reagents

i) Absolute alcohol
ii) 5% mercuric chloride solution
iii) 5% cupric sulphate solution
iv) 5% lead acetate
v) 5% ammonium sulphate

Tests for Steroids:

1. **Salkowski Reaction**: To 2 ml of extract, 2 ml chloroform and 2 ml concentrated H_2SO_4 was added. Shook well, whether chloroform layer appear red and acid layer show greenish yellow fluorescence is observe.
2. **Libermann-Burchard Reaction**: Mix 2ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops of concentrated H_2SO_4 from the side of test tube, observe for first red, then blue and finally green colour.
3. **Libermann reaction**: Mix 3 ml extract with 3 ml acetic anhydride. Heat and cool. Add few drops of concentrated H2SO4, observe for blue colour.

Tests for Alkaloids:

1. **Dragendroff's test**: To 2-3 ml of filtrate, few drop of Dragendroff's reagent is added. Observe for orange brown precipitate.
2. **Mayer's test**: To2-3 ml of filtrate, few drops of Mayer's reagent are added. Observe for precipitate.
3. **Hager's test**: To 2-3 ml of filtrate, few drops of Hager's reagent added. Observe for yellow precipitate.
4. **Wagner's test**: To 2-3 ml of filtrate, few drops of Wagner's reagent is added. Observe for reddish brown precipitate.

Tests for Tannins and Phenolic Compounds:

To 2-3 ml of the test solution, few drops of the following solutions is added and observed for the reaction formed.

1. 5% Ferric chloride solution: - Deep blue-black colour is formed.
2. Lead acetate solution: - White precipitate occurs.
3. Gelatin solution: - White precipitate occurs.
4. Bromine water: - Decolourization of bromine water takes place.
5. Acetic acid solution: - Red colour solution is formed.
6. KMnO4:- Decolourization of sample takes place.

Tests for Flavonoids:

1. Shinoda test:To the dried powder or extract, add 5 ml 95% ethanol, few drops of concentrated HCl and 0.5 g of magnesium turnings. Pink colour appears.
2. To the small quantity of residue, lead acetate solution is added. Observe for yellow coloured precipitate.
3. Addition of increasing amount of sodium hydroxide to the residue, yellow colour occurs and latter it decolorize on addition of acid.
4. Ferric chloride test:To the test solution, add few drops of ferric chloride solution observe for intense green colour.

Tests for Glycosides:

General test for Glycosides: Part A:To 2-3 ml of extract is mixed with dil. H_2SO_4 and heated on a water bath for 1-2 min. Neutralize with 10% NaOH, check with litmus paper and to resulting solution add Fehling's A & B. Increased red precipitate in this case shows glycosides are present.

Part B:To 2-3 ml of extract is mixed with water and heated. After cooling, NaOH is added for neutralization along with equal quantity of water. To the resulting solution Fehling's A & B is add. Increased red precipitate in this case show glycosides are absent. Compare Part A and B.

Tests for Cardiac Glycosides:

1. **Baljet's test**: The test solution on addition with sodium picrate forms yellow to orange colour.
2. **Legal's test**: To aqueous or alcoholic test solution, add 1 ml pyridine and 1 ml sodium

nitroprusside, observed for pink to red colour.

3. **Keller Killiani test**: To 2 ml of extract, few drops of glacial acetic acid, one drop of 5% $FeCl_3$ and concentrated H_2SO_4 is added. Observe for reddish brown colour at the junction of two liquids and upper layers turns to be bluish green.

4. **Libermann's test**: 3 ml of extract is mixed with 3 ml of acetic anhydride. Heat the solution and then cool and add few drops of concentrated H_2SO_4. Observed for blue colour formation.

Tests for Saponins:

1. **Foam test**: The extract or dry powder is shaken vigorously with water. Persistent foam is observed.

2. **Haemolytic test**: Add the test solution to one drop of blood placed on glass slide. Haemolytic zone is observed.

3. Isolation and characterization of Bioactives:

Based upon the preliminary phytochemical screening results, the ethanolic extract (EE) was subjected to column chromatography to isolate the various fractions of compounds. The elution was carriedout into the column containing activated silica gel-C (60 - 120 mesh), using highly non polar and polar solvents such as petroleum ether (PE), Benzene (BZ), Chloroform (CF), methanol (ME) and Double distilled water (H_2O) with increase in their polarity and percentages like 100% PE, PE : BZ (95:05 - 05:95), 100% BZ, BZ : CF (95:05 - 05:95), 100% CF, CF : ME (95:05 - 05:95), 100% ME and ME: H_2O (95:05 - 05:95). The various fractions obtained were collected separately into the beakers with the volume of 25 ml each elutent and immediately checked on TLC to identify the presence of single spot crystalline iodine was used for detecting the spot. The fractions which showed single spots on TLC plates were evaporated under reduced pressure and again repurified by eluting through the freshly prepared column using the respective solvent ratio. The purified compounds were subjected for IR, ^1H NMR and GC-MS for spectral analysis for the characterization studies.

Fig: Column Chromatogram

3.1 Anthelmintic activity:

Ethanolic and aqueous extracts of the leaves of *Boswelliaserrata*were investigated for their anthelmintic activity against *Pheretimaposthuma*. Various concentrations (25-10mg/ml) of both extracts were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Piperazine citrate was included as standard drug and normal saline as control[87], with minor modifications. The assay was performed on adult Indian earthworm, *Pheretimaposthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings[88, 89, 90, 91]. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro[92,93]. Indian adult earthworms (*Pheretimaposthuma*) collected from moist soil and washed with normal saline to remove complete faecal matter were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1 - 0.2 cm in width were used for all the experimental protocol. The earth worms were divided into ten groups (n=6). Group I - control (0.9% normal saline), Group II - standard piperazine citrate (15mg/ml), Group III – EE (25mg/ml), Group IV - EE (50mg/ml) Group V- EE (75mg/ml), Group VI - EE (100 mg/ml) and Group VII - AE (25mg/ml), Group VIII - AE (50mg/ml), Group IX - AE (75mg/ml) and Group X - AE (100mg/ml). Both the test samples and the standard drug are dissolved in 0.9% normal

saline. Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colours.

3.2 Acute (Oral) Toxicity Studies:

Acute toxicity studies for aqueous, ethanolic extract and isolated compound of *Boswelliaserrata* were conducted as per OECD guideline 420 (modified, adopted 23rd march 2006) using Albino Wister mice. Each animal was administered aqueous, ethanolic and isolated compound solution by oral route. The test procedure minimizes the number of animals required to estimate the oral acute toxicity of a chemical and in addition estimation of LD_{50}, confidence intervals. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.

3.3 Antiulcer activity by Pylorus ligation method :

Albino wistar rats of either sex weighing between (150-200gms) were divided into six groups of six animals in group.

Group-I – Control (2% gum acacia)
Group-II – Standard drug (Lansoprazole 8mg/kg in 2% gum acacia).
Group-III –AE - Aqueous extract (250mg/kg).
Group-IV – EE- Ethanolic extracts (250mg/kg).
Group-V – IC- (Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol) $_n$ (40 mg/kg)

In this method albino rats were fasted in individual cages for 24 hr. care was taken to avoid coprophagy. Extracts or isolated compound or standard drug or control vehicle was administered 30min. prior to pyloric ligation. Under light ether anaesthesia, give an incision of 1cm long in the abdomen just below the sternum. Expose the stomach pass a thread around the pyloric sphincter and apply a tight knot. While putting the knot care was taken so that no blood vessels are tied along the knot. The abdomen was sutured clean the skin from any blood spots and bleeding. Apply collodion over the wound. At the end of 4 hr. after ligation the animals were sacrificed with excess of anaesthetic ether. Open the abdomen and tie the oesophageal end (cardiac end) of the stomach. Cut and removed the entire stomach from the body of the animal. Gastric juice was collected into graduated centrifugation tube and was centrifuged at 1000 rpm for 10 min. and gastric volume was noted. The p^H of the gastric juice was recorded by P^H meter. Then the centrifuged supernatant contents were subjected to analysis for free and total acidity. Open the stomach along the greater curvature and washed with running water to see for ulcers in glandular portion of the stomach.

The number of ulcers per stomach was noted and severity of the ulcers of the ulcers scored microscopically with the help of hand lens (10X) and scoring was done as following.

0 = normal stomach.
0.5 = red coloration.
1.0 = spot ulcers.
1.5 = hemorrhagic streaks.
2.0 = ulcer > 3 but < 5.
3.0 = ulcer > 5

Mean ulcer score for each animal is expressed as ulcer index. The percentage protection was calculated using the formula,

Percentage protection = $100 - U_t/U_c \times 100$

Where, U_t = ulcer index of treated group.
U_c = ulcer index of control group.

3.4 Determination of free acidity and total acidity:

One ml of gastric juice was pipetted into 100ml conical flask, added 2 to 3 drops of Topfer's reagent and titrated with 0.01N NaOH until all traces of red colour disappears and the colour of the solution turns to yellowish orange. The volume of the alkali added was noted. This volume corresponds to free acidity. Then 2 to 3 drops of phenolphthalein solution was added and titration was continued until define red tinge reappears. Again the total volume of alkali added was noted. The volume corresponds to total acidity.

Acidity was calculated by following formula:

$$\text{Acidity} = \frac{\text{Volume of NaOH X Normality of NaOH X 100}}{0.1} \text{ meq / L / 100 gm}$$

In this method following parameters was studied-

1. P^H of gastric juice.
2. Volume of gastric secretion.
3. Free acidity.
4. Total acidity
5. Ulcer index.
6. % protection.

4. Histopathological evaluation:

The stomachs were immersed in 10 % formalin solution for Histopathological examination. These tissues were processed and embedded in paraffin wax. The central part of damaged or ulcerated tissue (if present) was cut on half along the long diameter. If the stomach was protected from the damage then the section was taken from basal part using a rotary microtome, sections of thickness of about 5 µm were cut and stained with haematoxylin and eosin. These were examined under the microscope for histopathological changes such as congestion, haemorrhage, necrosis, inflammation, Infiltration, erosion and ulcer and photographs were taken.

4.1 Statistical analysis

Results were expressed as mean ± SEM, (n=5). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Dennett's 't' test P value less than <0.05 was considered to be statistically significant. *P<0.05, **<0.01 and ***<0.001, when compared with control and toxicant group as applicable.

5. Results and Disscussion:

Table : Nature and yield of *Boswelliaserrata* leaves extracts

Sl. No	Extracts	Colour	Nature	% Yield (g)
1	PEE	Dark Greenish	Solid	6.5
2	CE	Brownish	Solid	3.5
3	EE	Brownish	Semi-solid	9.0
4	AE	Brownish	Semi-solid	7.0

Key: PEE-Petroleum ether extract, CE-Chloroform extract, EE-Ethanol extract and AE-

The various extracts obtained from sequential extraction of *Boswelliaserrata* leaves were assayed for the production of yield and detecting the presence of active constituents present in them. Further after preliminary screening, the bioactive were isolated, characterized and pharmacologically screened.

Aqueous extract.

Preliminary phytochemical analysis of *Boswelliaserrata* leaves extracts

Tests	PEE	CE	EE	AE
Test for carbohydrates				
a)Molish's test	-	+	+	-
b)Iodine test	-	-	-	-
c)Fehling's test	-	-	+	-
d)Benedict's reagent	-	+	-	-
e)Selwinoff's test	-	-	-	-
Test for proteins				
a) Biuret test	-	+	+	-
b)Ninhydrin reagent	-	+	+	-
c)Biuret test	-	-	-	-
d) Millon's test	-	-	-	-
e) Xanthoprotein test	-	-	-	-
f) Precipitation test	-	-	+	-
Steroids				
a) Salkowski reaction	+	-	-	-
b)Libermann buchard's reaction	+	+	-	-
c) Libermann reaction	+	-	-	-
Test for Alkaloids				
a) Dragendroff's test	-	+	-	-
b) Hager's test	+	+	+	-
c) Mayer's test	-	+	-	-
d) Wager's test	-	+	+	+
Test for Tannins	-	+	+	+
a) 5% $FeCl_3$	-	-	+	-
b) Lead acetate	-	+	-	+
c) Gelatine solution	-	-	+	+
d) Bromine water	-	-	-	-
e) Acetic acid	-	-	+	+
k) $KMnO_4$				
Test for Flavonoids	-	-	+	-
a) Shinoda test	-	-	+	+
b) lead acetate test	-	-	+	+
c) Sodium hydroxide test	-	-	+	+
d)Ferric chloride test				
Test for Glycosides	-	-	+	-
a)Legal test	-	+	+	+
b) Baljet test	-	+	+	-
b)keller –killiani	-	-	-	+
c)Borntrager's test				
Test for Saponins	+	+	+	+
a) Foam test	-	-	+	+

Key: PEE-Petroleum ether extract, CE-Chloroform extract, EE-Ethanol extract and AE- Aqueous extract.

Anthelmintic Activity

Based upon the preliminary phytochemical screening, both the AE and EE were subjected for antihelmintic activity compared with standard drug, Piperazine citrate. The result obtained in

this study is based upon the observation made on helimentic paralysis and death at different time intervals. Table 6 shows the time taken for the paralysis and death of helminths by the action of both III (EE) and IV (AE). It can be seen that the highest activity was exhibited by the concentration greater than50mg/ml, but the highest activity exhibited at 100 mg/ml within the time span of 30min. These test samples were compared with group II (Piperazine Citrate), at the lowest concentration of 15mg/ml
depicts the paralytic condition and the death of the helminths at different concentration levels compared with the normal and standard drug.

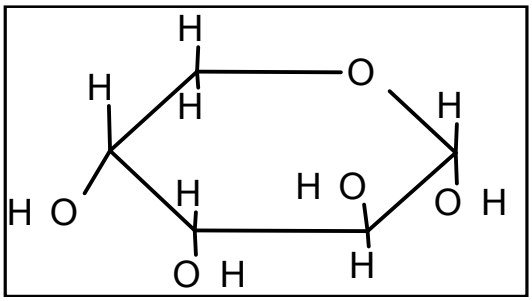
In vitro evaluation of crude extract of *Boswelliaserrata*by anthelmintic activity

Group	% Concentration (mg/ml)	Paralysis (min)	Death (min)
I (Control)	-	-	-
II (Piperazine citrate)	15	38±0.31	56±0.34
III (EE)	25 50 75 100	40±0.36 29±0.42 24±0.57 20±0.71	58±0.11 41±0.35 32±0.42 27±0.20
IV (AE)	25 50 75 100	42±0.62 30±0.04 27±0.65 21±0.42	67±0.21 48±0.53 36±0.45 24±0.33

Key: PEE-Petroleum ether extract, CE-Chloroform extract, EE-Ethanol extract and AE-Aqueous extract.
All Values are expressed as Mean± SEM.

Isolation and Characterization of bioactive constituent

Based upon the preliminary screening by the phytochemical and the anthelmintic studies, the ethanolic extract subjected through column chromatography for the isolation of pure compound was successfully carriedout. Various eluents collected at a flow rate of 5 ml/min, subjected for TLC separation to obtain a single spot made to analyze the compound obtained from the CH:Me (20:80) ratio for various spectral studies.
The IR spectrum of compound showed the broad and intense peak at 3386 cm^{-1} and a peak at 1701cm^{-1} and 1652cm^{-1} was also detected. Aromatic C-H absorption peak were not found, but peaks at 2926cm^{-1} and 2854cm^{-1} shows the presence of allyl C-H bond. The broad intense peak, 3686cm^{-1} suggests that there may be number of secondary –OH group along with primary –OH. A peak at 1701cm^{-1} may be due to the lactone rings. The H^1 NMR spectrum exhibited absorption peaks at 1.25 ḍ and 1.5 ḍ and 2.2 ḍ corresponding to CH-CH_2 protons present in the molecule. The sample gave number of fragment ions above 300 m/z. These data suggest that the molecule under the investigation may be a polysaccharide containing number of primary and secondary hydroxyl groups and lactone ring systems. There is no aromatic ring systems in the molecule due to the absence of aromatic structure in H^1 NMR system, but only absorption peaks corresponding to CH_2-CH protons has been observed at 1.3 ḍ and 1.7 ḍ and 2.2 ḍ. In the mass spectral studies, the polysaccharide fragments gave rise to monomers corresponding to pentose sugars. This is for the substantiated by the presence of a base peak at 73 m /z, which is shown in the fragmentation of the molecule. It is possible to get the mass of the various fragments if recorded lesser electronic volt (eV).



Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol

Anthelmintic effects of ethanolic and aqueous extracts of *Boswelliaserrata*

Acute toxicity (LD_{50}) studies

Acute toxicity studies based up on the continuous monitoring and observation for 72 hours as per the OECD guidelines 420, the oral acute toxicity studies revealed the non-toxic nature of the test samples of Boswelliaserrata. During this study the effective dose (ED50) was notified as 250mg/kg body weight with both crude aqueous and ethanolic extracts, and 40mg/kg in case of (Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol)$_n$. No lethality of animals was found up to 2000mg/kg body weight. Hence the LD_{50} study was stopped at this dose.

Antiulcer activity

Based upon the oral acute toxicity studies, an effective dose of 250mg/kg of both the EE and AE, and 40 mg/kg in case of the isolated compound, (Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol) $_n$ was administered orally. The test samples were compared with the standard, Lansoprazole (8mg/kg).

Table 7 summarizes the effect of *Boswelliaserrata* on the volume of gastric secretion, free acidity, total acidity and pH following pylorus ligation in rats. In case of volume of gastric secretion, a significant reduction in the Volume of gastric juice was exhibited in Group V with $P< 0.001$. While Group III and IV showed moderate reduction in the volume of gastric content with $P< 0.05$. In case of free acidity studies, a significant reduction in free acidity was exhibited in Group IV and V with $P< 0.001$. But lesser significance of $P<0.05$ was found in Group III. In case of total acidity assay, a significant reduction in total acidity was seen in Group III and V with $P < 0.001$. Here the reduction in total acidity in Group V (IC) was greater than Group II (Lansoprazole). Group IV showed least significant, $P < 0.05$. The acidic and basic balance plays an important role in antiulcer effect. In case of ulceration there is increase in H^+ ion concentration. The case of effect of P^H studies, there was significant reduction of H^+ ion concentration in Group III and V with $P < 0.001$. No significance was seen in case of Group IV. All the parameters studied in the gastric secretion produced due to the pylorus ligation in rats were compared with Group II, Lansoprazole.

Table: Effect of *Boswelliaserrata*on gastric secretion following pyloric ligation in rats

Group	Treatment	Vol. Of Gastric Juice (Ml)	Free Acidity (meq/L) 100gm	Total Acidity (meq/L) 100gm	pH
I	Control	6.66 ± 0.084	76.78 ± 0.21	94.50 ± 3.56	2.56 ± 0.032
II	Lansoprazole (8mg/kg)	2.89 ± 0.064***	27.99 ± 0.188***	34.00 ± 1.89***	5.71 ± 0.041***
III	EE (250mg/kg)	4.79 ± 0.016**	45.25 ± 0.107*	40.83 ± 1.35**	4.01 ± 0.014**
IV	AE (250mg/kg)	5.27 ± 0.034*	38.25 ± 0.246**	64.33 ± 2.6*	3.02 ± 0.07*
V	IC (40mg/kg)	3.01 ± 0.042***	35.85 ± 0.0611***	32.83 ± 2.02***	4.95 ± 0.11***

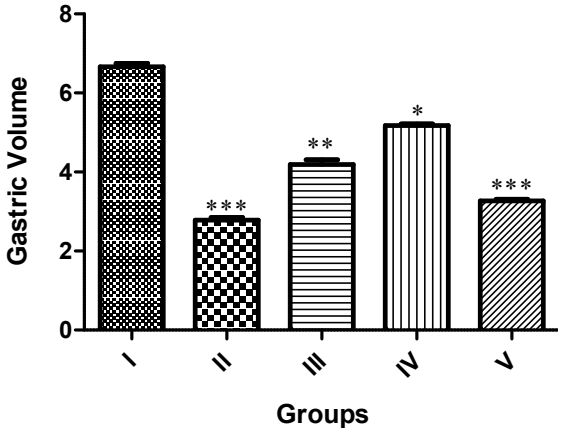
Key: EE-Ethanol extract, AE-Aqueous extract and IC-(Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol) $_n$
All Values are expressed as Mean± SEM; *P < 0.05, **P < 0.01 and ***P < 0.001 compared with Control.

Table 8 shows the effect of *Boswelliaserrata* on pylorus ligation induced ulceration in rats. In this assay, the ulcer index and percentage of ulcer protection was found. It could be noted that Group V showed significant reduction in the ulcer index with $P< 0.001$ and $P < 0.01$ in case of Group III and IV compared to Group II, Lansoprazole.

Fig 10 – 13 shows the graphical illustration from the data represented in table 7, showing the effect of *Boswelliaserrata* on gastric secretion and fig 14 illustrated from table 8 represents the effect of Boswelliaserrata on ulcer index in pylorus ligation in rats.

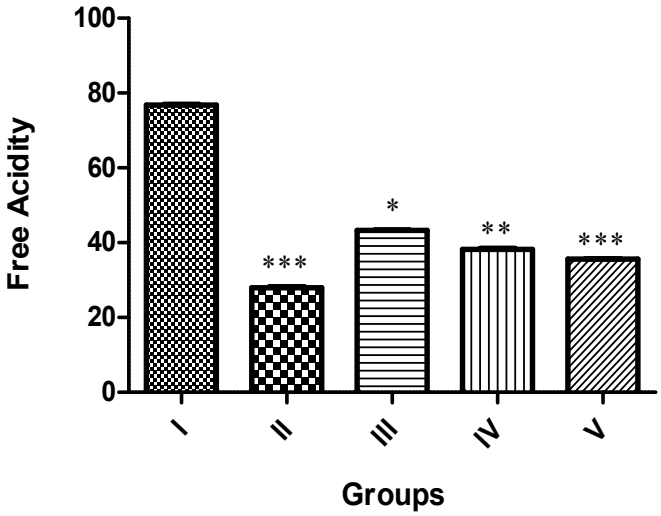
Thus, the antiulcer activity exhibited by the all the three test samples that is EE, AE and IC when compared to Lansoprazole exhibited a significant reduction in the ulcers showing the potential healing property of *Boswelliaserrata*.

Effect of *Boswelliaserrata*on volume of gastric juice following pyloric ligation in rats



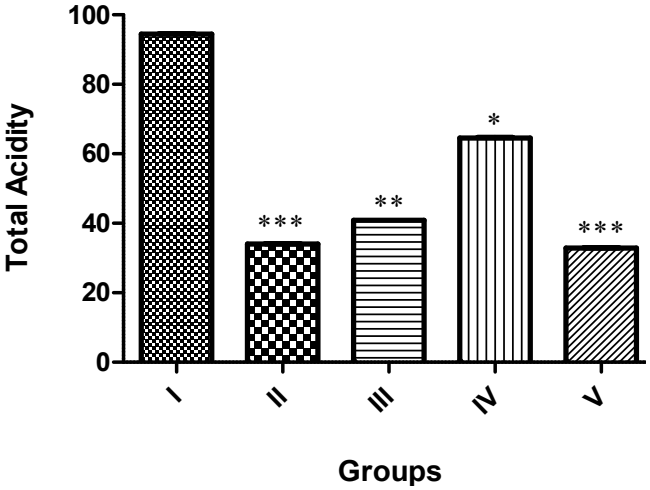
Key Groups:
I– Control
II- Standard
III– Ethanolicextract (EE)
IV– Aqueous extract (AE)
V- (Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol)

Effect of *Boswelliaserrata* on free acidity following pyloric ligation in rats



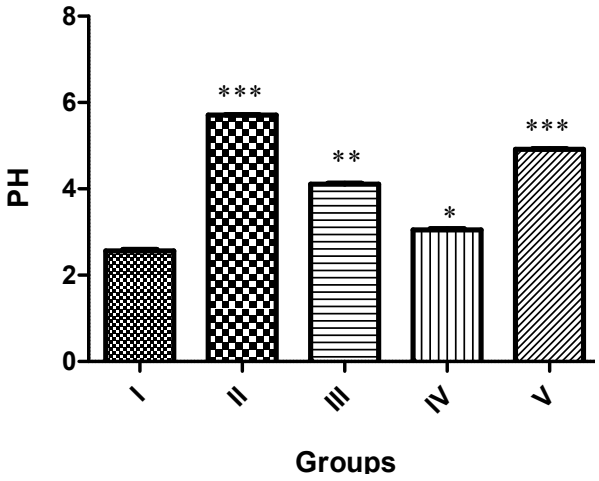
Key Groups:
I– Control
II- Standard
III– Ethanolicextract (EE)
IV– Aqueous extract (AE)
V- (Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol) $_n$

Effect of *Boswelliaserrata* on total acidity following pyloric ligation in rats



Key Groups:
I– Control
II- Standard
III– Ethanolicextract (EE)
IV– Aqueous extract (AE)
V- (Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol) $_n$

Effect of *Boswelliaserrata* on pH in pyloric ligation in rats



Key Groups:
I– Control
II- Standard
III– Ethanolicextract (EE)
IV– Aqueous extract (AE)
V- (Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol) $_n$

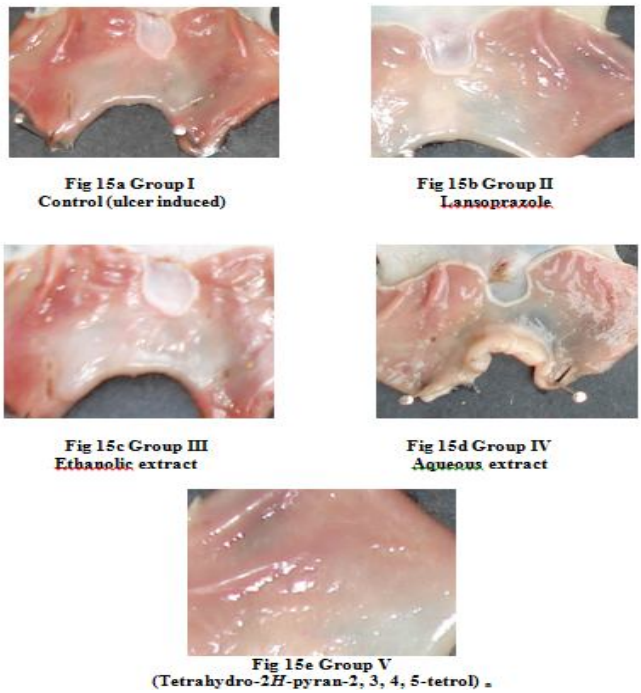


Fig 15a Group I
Control (ulcer induced)

Fig 15b Group II
Lansoprazole

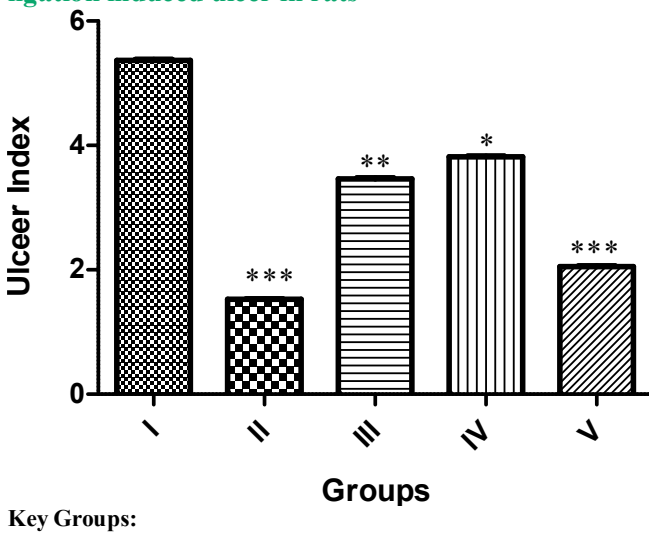
Fig 15c Group III
Ethanolic extract

Fig 15d Group IV
Aqueous extract

Fig 15e Group V
(Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol) $_n$

Effect of *Boswelliaserrata* ulcer index pylorus ligation induced ulcer in rats

Effect of *Boswelliaserrata* on ulcer index pylorus ligation induced ulcer in rats



Key Groups:
I– Control
II- Standard
III– Ethanolicextract (EE)
IV– Aqueous extract (AE)
V- (Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol) $_n$

Histopathological studies in pylorus ligation induced ulcer in rat.

The Histopathological studies in pylorus ligation induced ulcer in rat as shown in Fig 16a-16e exemplifies the comparative histopathological studies in various groups based upon the accessing the presence or absence of the redness, infiltration, congestion, hemorrhagic sticks, inflammation, necrosis and dilation of blood vessels.In case of Group I, the ulcer induced without treatment sample shows the presence of redness, infiltration, congestion, hemorrhagic sticks, inflammation, necrosis and dilation of blood vessels in the mucosa of the tissue. Group II, when treated with Lansoprazole shows the mild redness, no inflammation and dilation of blood vessels in the mucosa. Group III, when treated with ethanolic extract, the mucosa showed the mild inflammation and congested blood vessels. Group IV, treated with aqueous extract exhibited fibrosis and mild inflammation in mucosa.

No inflammation and no congestion of blood vessels was seen in Group V, treated with (Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol) $_n$.

The yield production and the nature of the drug helps to study the safety and efficacy of the crude drug analysis and the formulations prescribed for the treatment of various illness. This helps in the analysis and standardization of natural products in the pharmaceutical care.

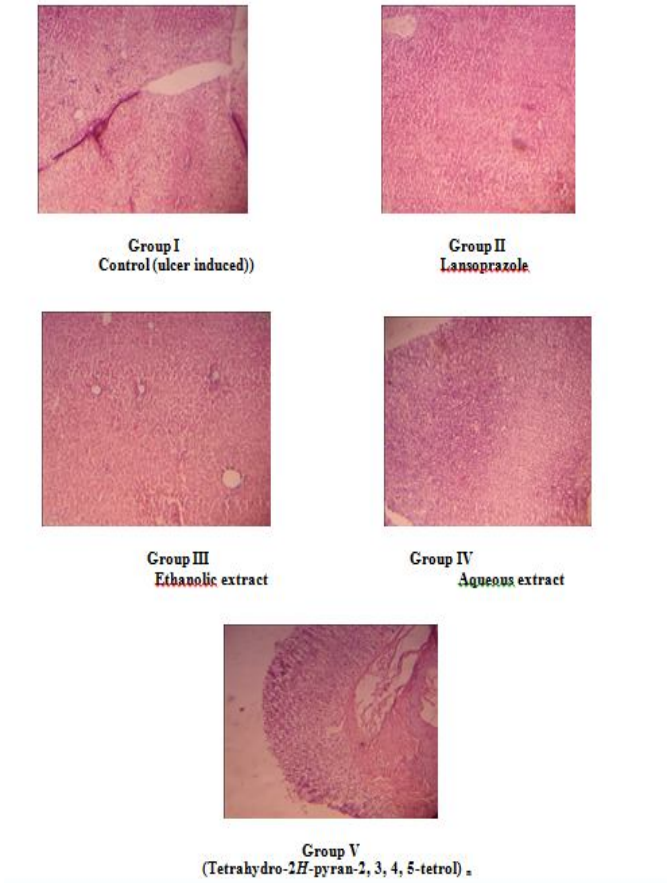
The table 5 summarizes the Preliminary phytochemical constituents that are present in *Boswelliaserrata* leaves extracts. Various primary and secondary metabolites such as carbohydrates, proteins, steroids, alkaloids, polyphenols like tannins, flavonoids, triterpenes and saponins has been detected in the preliminary phytochemical screening of the crude extracts of *Boswelliaserrata*.

These bioactives synthesized during various metabolic pathways of the plant helps in curing the diseases without side effects or least effects. They resembles to various synthetic drugs as a structural analogues that are being used in pharmaceutical products. To assay these phytoconstituents a preliminary antihelmintic screening carried out showed the effective results from the two test samples i.e. ethanolic extract (EE) and aqueous extracts (AE) use for antihelmintic screening purpose.

The EE and AE used to evaluate anthelmintic activity, showed variable effective results at different concentrations with the mean time values. As in table 6 the significant result was exhibited in the concentration 50, 75 and 100 mg/ml of both extracts when compared with the standard drug Piperazine citrate. The activity was assayed by the observation of the helminthes with the paralysis and the death at different concentrations and varying time intervals in each case.

Table 1: Drugs obtained from herbs and plants, source, therapeutic activity and their synthetic or semi-synthetic analogs.

Sl. No	Drugs from herbs and plants	Source	Therapeutic activity	Synthetic or synthetic analogs
1	Atropine	*Atropa belladonna*	Antimuscarinics	Dicyclomine HCl, Hyoscinebutylbromide
2	Benzylepenicillin	*Penicillin chrysogenum*	Antibiotic	Ampicillin, amoxicillin.
3	Codeine	*Papaversomniferum*	Analgesic	Nolorphine, mepiridine.
4	Camptothecine	*Camptothecaaccumnata*	Anticancer	10-hydroxycaptothecine, aminocamptothecine, topotecan, ironotecan.
5	Digoxin	*Digitalis lanata*	Cardiovascular	-
6	Ephedrine	*Ephedra vulgaris*	Anti-asthma	Salbutamol, salmeterol.
7	Lovastatin	*Aspergillusterrus*	hypercholesterolemia	Pravastatin.
8	Morphine	*Papaversomniferum*	Analgesic	Heroine, naloxane, phthadine.
9	Podophyllotoxine	*Podophillumpettatum*	Anticancer	Etopside, toniposide.
10	Quinine	*Cinchona succirubra*	Antimalerial	Chloroquine, meploquinine, pamaquine, premquine.
11	Reserpine	*Rawolfiaserpentina*	Hypotension and anticholinergic	-
12	Tubacurarine	*Tubocurare*	Neuromuscular blocking agent	Decamethoxium, soxamethorium.
13	Taxol	*Taxusbaccata*	Anticancer	-
14	Teprotide	*Bathrops*	Antihypertensive	Captopril, enalapril, lisinopril.
15	Vinblastin, vincristine	*Cathranthusroseus*	Anticancer	Vindesine.



However, when observed the response of worms in case of paralysis, there was significant

variation among the results produced by both the extracts. The EE showed more significant effect on paralyzing the worms, in terms of paralysistime, at every concentration compared to that of AE. The effect of extracts on the paralysis (or) helminthiasis of the worm, as in table-6 may be indicated. This may be due to the presence of active phytoconstituents and there potentiality for the antihelmintic activity. The polyphenols such as tannins in both the extracts may be one of the major causes for helminthiasis[96, 97]. These Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation or, binds to the glycoprotein on the cuticle of parasite[98], and cause death. Coming to the chemistry of nematode surface, it is a collagen rich extracellular matrix (ECM) providing protective cuticle that forms exoskeleton, and is critical for viability, the collagen is a class of proteins that are modified by a range co-and post –translational modification prior to assembly into higher order complexes (or) ECMS[99]. The mammalian skin also consists largely of collagen in the form of fibrous bundles. In leather making industry, vegetable tannins are commonly used in the tanning operation of leather processing that imparts stability to collagen of skin matrix through its reactivity and hence make the collagen molecule aggregate into fibres. This results in the loss of flexibility in the collagen matrix and gain of mechanical property with improved resistance to the thermal (or) microbial/enzymatic attack. Similar kind of reaction is expected to take place between the nematode cuticle (the earth worm) and the tannin of *Enicostemmalittorale*, possibly by linking through hydrogen bonding, as proposed in this study. This form of reactivity brings toughness in the skin and hence the worms become immobile and non-functional leading to paralysis followed by death.

With the confirmed preliminary screening by phytochemical analysis and antihelmintic activity carriedout, the ethanolic extract subjected for column chromatography has led with the successful isolation of various chemical constituents. Among them, the pure compound isolated from the elution of chloroform: methanol in the ratio 20:80, checked on TLC for the presence of single spot was further assayed through various spectral analysis such as IR, H^1 NMR and GCMS. The interpretation of the data obtained by the IR studies reveals that peaks obtained at different wavelengths as described in results chapter, clearly signifies the presence of allyl C-H bond, the number of both the primary and secondary –OH groups, and the lactone ring system present in the molecule obtained. In case of H^1NMR spectra, various absorption peaks obtained shows the presence of CH-CH2 protons in the molecules with the number of fragment ion above 300m/z. The spectral data do not contain aromatic ring system but the presence of polysaccharide with the primary and secondary –OH groups. The mass spectral studies signified the presence of various polysaccharide fragments giving rise to pentose monomer which has been substantiated at the ionic concentration with the base peak of 73m/z.

Thus with spectral analysis of IR, H^1 NMR and GCMS, has paved a tentative structural illustration of the pure compound (Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol) $_n$ and its monomer, a pentose called Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol. Thus this pure compound has been successfully isolated and subjected for biological screening to check its antiulcer property as per the tribal practice is concerned with the administration of crude drug of*Boswelliaserrata* without proper scientific investigation carriedout.

It is generally accepted that gastric ulcers results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism[100]. Peptic ulcer results due to overproduction of gastric acid (or) decrease in gastric mucosal production. Further, the role of free radicals is also reported in the indication of ulcers. Prostaglandins (PG) offer protection to duodenum through both increases in mucosal resistance as well as decrease in aggressive factors, mainly acid and Pepsin[101]. Pylorus ligation induced ulcers are due to auto digestion at the gastric mucosa and breakdown of the

gastric mucosal barrier [102]. In case of pyloric ligation, ulcer formation is mainly due to the stasis at the gastric juice and stress[103].

Prior to the biological activity, the effectiveness and the lethality of the drug has to be assayed to avoid unnecessary cruelty, trial and error methods carriedout on the experimental animals. Hence based upon the OECD 420, the oral acute toxicity studies carriedout has successfully fixed an effective dose of 250mg/kg of both the EE and AE, and 40 mg/kg in case of the isolated compound, (Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol) $_n$.

Table 7 summarizes the effect of *Boswelliaserrata* on the volume of gastric secretion, free acidity, total acidity and pH following pylorus ligation in rats. In case of volume of gastric secretion, a significant reduction in the Volume of gastric juice was exhibited in Group V ($P < 0.001$) and ($P< 0.05$) was seen in Group III and IV. In the free acidity parameter, a significant reduction in free acidity was exhibited in Group IV and V ($P< 0.001$) and ($P<0.05$) in Group III. In case of total acidity assay, a significant reduction in total acidity was seen in Group III and V ($P < 0.001$) while Group IV showed least significant, $P < 0.05$. The acidic and basic balance plays an important role in antiulcer effect. In case of ulceration there is increase in H^+ ion concentration. The case of effect of P^H studies, there was significant reduction of H^+ ion concentration in Group III and V ($P < 0.001$). No significant was found in Group IV. All the parameters studied in the gastric secretion produced due to the pylorus ligation in rats were compared with Group II, Lansoprazole.

Table 8 shows the effect of *Boswelliaserrata* on pylorus ligation induced ulceration in rats. In this assay, the ulcer index and percentage of ulcer protection was found. The ulcer index in Group V ($P< 0.001$) and Group III and IV ($P < 0.01$) showed significant reduction compared to Group II, Lansoprazole.

The anti-secretory activity of the extracts noticed in pylorus ligation induced ulcer model, which decreased in the volume of gastric juice, reduction in free and total acidity and pH in the animals treated with the test samples may be due to the presence of potential and curative phytoconstituents present in *Boswelliaserrata* and was found to be devoid of ulcerogenic potential. Literature review suggests that the majority of the antiulcer activity proposed in various models of antiulcer studies by pylorus ligation, aspirin and ethanol induced ulcer models has been attributed with antiulcer property due to the various active constituents like flavonoids, tannins, terpenes, steroids, Saponins, alkaloids and glycosides responsible as antiulcerogenic agents[83,104]. Significant results without any toxicity has also been exhibited in the polyherbalformulation[76,77,78].

The Histopathological studies in pylorus ligation induced ulcer in rats as shown in Fig 16 exemplifies the comparative histopathological studies in various groups based upon the accessing the presence or absence of the redness, infiltration, congestion, hemorrhagic sticks, inflammation, necrosis and dilation of blood vessels. In fig 16a, Group I showed the ulcerogenic wounds with the presence of redness, infiltration, congestion, hemorrhagic sticks, inflammation, necrosis and dilation of blood vessels in the mucosa of the tissue. This is due to the interruption of the ulcerogenic agents on the parietal cells of gastric mucosal layer causing the disruption of the structural and functional aspects of gastric cells leading to the abnormal production of the gastric contents with decrease in pH and inhibited enzymatic activity and auto destruction of gastric cells. Group II, when treated with Lansoprazole shows the mild redness, no inflammation and dilation of blood vessels in the mucosa. Group III, when treated with ethanolic extract, the mucosa showed the mild inflammation and congested blood vessels. Group IV, treated with aqueous extract exhibited fibrosis and mild inflammation in mucosa. No inflammation and no congestion of blood vessels was seen in Group V, treated with (Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol) $_n$. Changes in the histopathological view in various groups treated with test samples may be attributed due to the dose dependence, mode of action, intensity and the potential efficacy of the drugs administered in

the ulcerogenic animals as shown in fig 16b – 16e.

Thus the *Boswelliaserrata* has been successfully screened with antiulcer activity possessing various phytoconstituents exhibiting the curative property and can be further analyzed for various pharmacological screening and formulations to produce the scientific evidence abbot the usage of the plants used by the non-registered practitioners just based upon the collection and compilation of tribal and folklore knowledge.

4. Conclussion:

Herbs are an integral part of nature containing natural substances that can promote good health. Natural product especially those derived from plant sources is gaining much interest for therapeutic use than that of the conventional ones. This is due to development of resistance and unwanted side effects. But natural product exhibit minimal resistance and negligible side effects though they are well tolerated. The isolation of biologically active phytoconstituents such as alkaloids, Quinine, serpentine, reserpine, narcotine, caffeine, nicotine, etc is due to the result of initial leads obtained from the traditional system of medicine. Explanation of chemical constituents of plants and pharmacological screening may provide us the basis for the developing the leads for the development of novel agents for curative purposes for treating diseases like diabetes, cancer, sexually transmitted diseases neurological and immunological disorders etc.

The investigation carriedout on *Boswelliaserrata*based upon the literature survey from the alternative system of medicine has made to establish the scientific basis of knowledge developed by the phytochemical and pharmacological screening. During this study various phytoconstituents were detected and screened successfully by the antihelmintic activity. This has led to isolate the pure compounds and study their biological activity. The isolated polysaccharide so called as (Tetrahydro-2*H*-pyran-2, 3, 4, 5-tetrol) $_n$ screened for antiulcer activity along with other crude extract exhibited significant reduction in antiulcer activity.

Thus it could be concluded that the *Boswelliaserrata*possess various bioactive compounds that can be used for the treatment of various diseases based upon the experimental evidences established in our studies. Furthermore, a detailed study needs to be carriedout to establish proper and complete scientific evidence to establish a novel drug formulation with least or no side effects. It is also mandatory to screen the isolated compounds with various pharmacological activities and the mechanisms of action of the drugs needs to be understood.

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