

Anhelmintic Activity of Ethanolic Extract of Citrus Limon Seeds

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ABSTRACT

The Citrus Limon seeds belonging to family Rutaceae are a common plant, native to Asia. Citrus food and derive product have beneficial impact to human health. More than a vitamin-

Crich fruits, Limon is powerful medicinal plant with numerous benefits that have been enjoyed for over 3000 years. The citrus Limon seeds are evaluated to determine the Anti-Helmenthic activity. The powdered seeds were extracted by maceration process using ethanol solvent. Phytochemical screening was carried out to check the presence of different

Phytochemical constituents like flavonoids, tannins, saponins, terpenoids and phenolic compounds. The results reveal a dose dependent increase in activity of the extracts at 50 mg/ml concentration. The extract at 50 mg/ml concentration. The extract at 50 mg/ml exhibited better activity than standard compound Albendazole

Keywords:

Anti-Helmenthic activity, Albendazole, Citrus Limon, Maceration, Phytochemical constituents.

I. INTRODUCTION

The word "helminth" comes from the Greek word "helminths" which means body. Gastrointestinal parasites are serious microorganisms in people, homegrown domesticated animals, and wild creatures. Almost all diseases because of helminths are by and large limited to tropical locales and cause colossal risk to well-being and add to the pervasiveness of undernourishment, eosinophilia and pneumonia.

They hurt the host by denying it food, causing blood misfortune, injury to organs, digestive or lymphatic hindrance, and emitting poisons. They are to blame for a lot of illness, like elephantiasis- causing lymphatic filariasis, river

blindness- causing onchocerciasis, schistosomiasis, weight loss, and poor fertility.

They are bad for your health and have pathological symptoms like diarrheal, droopiness, emaciation and anaemia.

One of the most prevalent infections worldwide, soil-transmitted helminth (STH) infections affect the poorest and most disadvantaged communities. Because they can be controlled or eradicated, these STHs are referred to as neglected tropical diseases (NTDs).

However, they cause a great deal of disability and suffering. It is an infestation caused by one or more round intestinal parasites, such as whipworms, hookworms, or Ascaris.

In endemic areas, these infections can affect the majority of the population, resulting in significant social and economic effects. Within an infected population, the prevalence of parasitic helminths typically follows a negative binomial distribution, with few individuals carrying high parasite burdens. Those individuals are most likely to fall ill and spread the disease to others in their community if they do not receive treatment.

Despite being the least common, tapeworms are to blame for the worst outcome, such as neurocysticercosis, which results in permanent brain damage and early death.

Other infections like Plasmodium falciparum, Human Immunodeficiency Virus (HIV), and Mycobacterium tuberculosis become more severe as a result of helminthiasis

1. CLASSIFICATION OF HELMINTHS:

The definitive classification is based on the external and internal morphology of egg, larval, and adult stages.

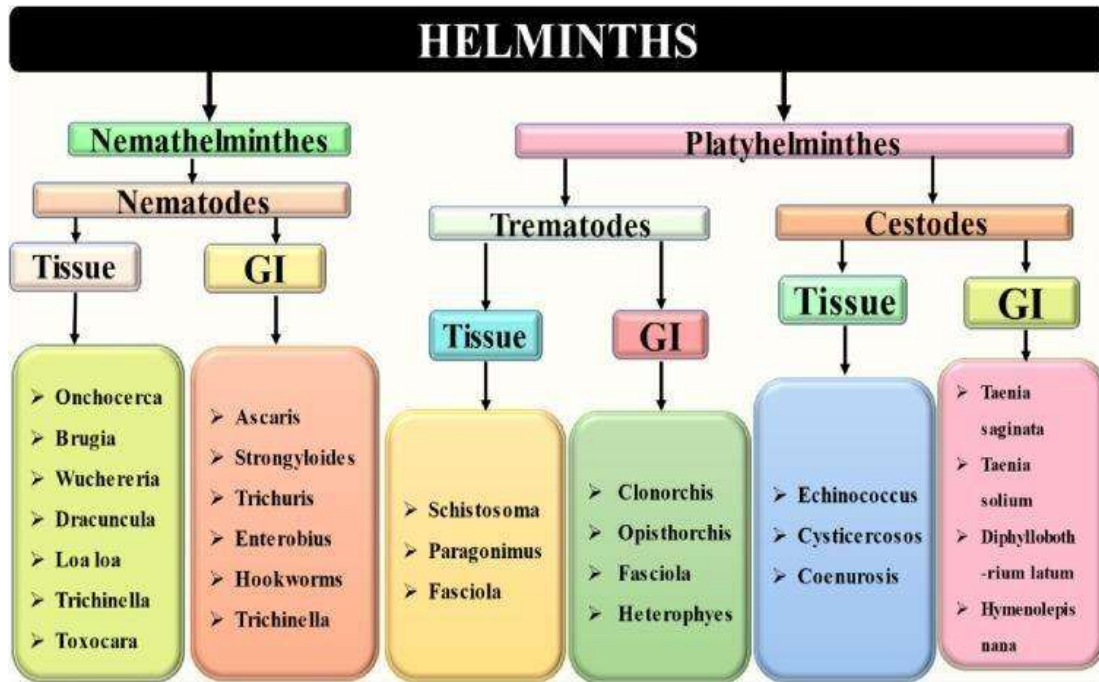


Fig: 1(classificationofanti helminths)

- Hookworms are known to cause chronic intestinal blood loss and can result in anaemia and chronic fatigue. Intestinal worms can cause diarrhea, abdominal pain, and a generalized feeling of discomfort, and weakness. In some people, intestinal worms do not cause any symptoms or they may be irregular. People are infected with Ascaris and whipworm when eggs are ingested. Hookworm eggs are not infective.
- About 40 million people are infected with food-borne trematodes. Food-borne trematodes are emerging in several countries due to the globalization of the food supply, increased international travel, population growth, pollution, ecological transformations, or poverty. Infections with food-borne trematodes cause inflammatory lesions and tissue damage, which can result in serious secondary complications such as cholangiocarcinoma in the case of infections with clonorchis sinensis and Opisthorchis viverrine.
- Roundworms are bisexual, cylindrical worms. They inhabit intestinal and extra-intestinal sites. In contrast to platyhelminths, nematodes are cylindrical rather than flattened; hence the common name roundworm. The body wall is composed of an outer cuticle that has a noncellular,

chemically complex structure, a thin hypodermis, and a musculature. The cuticle in some species has longitudinal ridges called alae. The bursa is a flap-like extension of the cuticle on the posterior end.

1. EPIDEMIOLOGY:

The World Health Organization (WHO) reveals that over 2 billion people, especially in developing countries, and particularly in children, are suffering from parasitic worm infections. Of which ascariasis is common, some other worms that cause parasitic infections are Hook worm, Trichuris trichura, and Hymenolepis nana etc. Helminthiasis is one of the most widespread infectious diseases affecting mostly children and pregnant women. In Helminthiasis this organism multiplies outside of the definitive host and has the unique ability to evade host immune defences, for reasons that are not fully understood. Helminthiasis is often chronic, possibly lasting an entire lifetime of the host. Infected host humans are divided into two categories or phyla.

1. Platyhelminthes (flatworms): In which A. Cestodes (tapeworms), B. Trematode (flukes) is included

2. Nematodes(roundworms): In which A.Roundworm,B.Hookworm, C.Pinworm,D.Whipwormareincluded

It is estimated that by the year 2025, about 57% of the population in developing countries will be influenced. Latest estimates indicate that 880 million children need treatment for these parasites. The population at risk in the WHO African Region is estimated at 350 million. A large part of the world's population is infected with one or more of these soil-transmitted helminths. Climate and soil structure are crucial determinantsof hookworm prevalence, as the parasite thrives in tropical and subtropical zones, where moisture and temperature are ideal for larval development outside the host. The different distribution of the various hookworm species is not absolute, with mixed infections often occurring in individuals. An estimated 438.9 million people (95% credible interval: 406.3–480.2 million) were infected with hookworm in 2010, with the largest concentration of hookworm cases in Southeast Asia, followed by sub-Saharan Africa. Hookworm infection tends to be more prevalent in rural areas, where the favourable tropical or subtropical ecologies converge with poverty and weak sanitary infrastructures.

2. ETIOLOGY:

- Intestinal parasite infections often cause morbidity and mortality, especially in children
- Poor hygiene of mother or caregiver is also one of the most important risk factors for soil-transmitted helminths infection in preschool children.

- The major risk factors of helminthiasis are rural areas, low socioeconomic status, poor sanitation, lack of health care, lack of education, inadequate dwelling conditions.
- Adult Ascaris is a long cylindrical worm, and its larvae can migrate into the pulmonary circulation.
- A. duodenale and N. americanus are retransmitted by penetration of the skin from where it goes into the lungs and crosses pulmonary capillaries to penetrate into alveolus and then to the intestine through the passing of larynx.
- Contact with contaminated feces.

3. TRANSMISSION & LIFECYCLE:

They are communicated by eggs present in human dung which thusly defile soil in regions where disinfection is poor. The roundworm (Ascaris lumbricoides), the whipworm (Trichuris trichura), and the hookworm (Necator americanus and Ancylostoma duodenale) are the main species that infect people.

Hookworms:

Hookworm eggs hatch in soil and rhabditiform (early) larvae moult twice before becoming infective. Larvae accumulate in soil or on grass awaiting exposure to human skin (often the hands, feet or buttocks) which they can penetrate. The larvae then make their way to the peripheral vasculature, where they are passively swept within the bloodstream, first to the right side of the heart and then to the pulmonary vasculature. Mature male and female hookworms produce as many as 10,000 eggs per day.

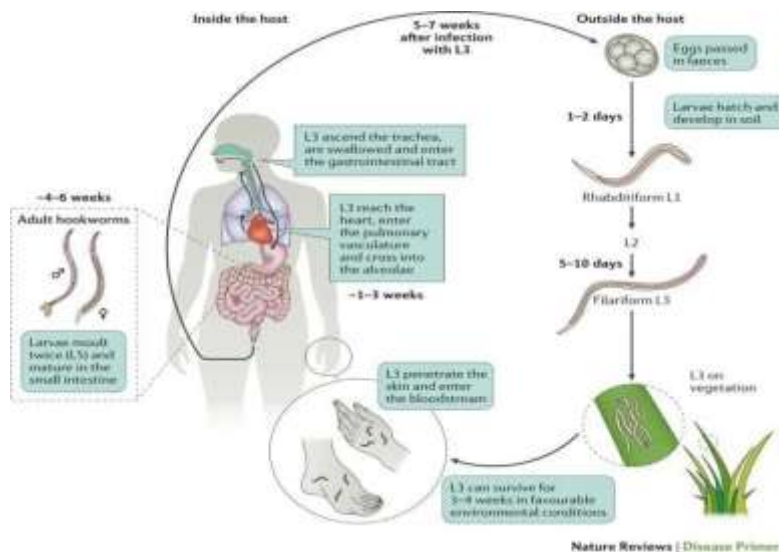
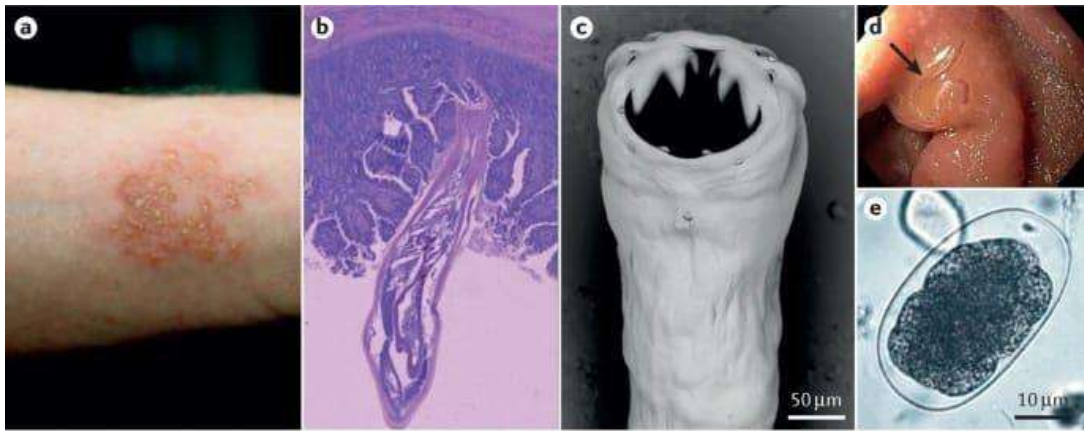


Fig:2 (Lifecycle of Hookworm)



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Fig:3 (Developmental stages of the intra-host phase of the hookworm lifecycle)

Whipworms:

Ingesting eggs or hatchlings causes whipworm. Consuming fruits and vegetables that have not been thoroughly washed, peeled, or cooked can result in infestation. After playing with even

eating dirt that has been contaminated, children are most likely to contract whipworm. Worms can continue to lay eggs for up to a year after being swallowed.

Whipworm Life Cycle

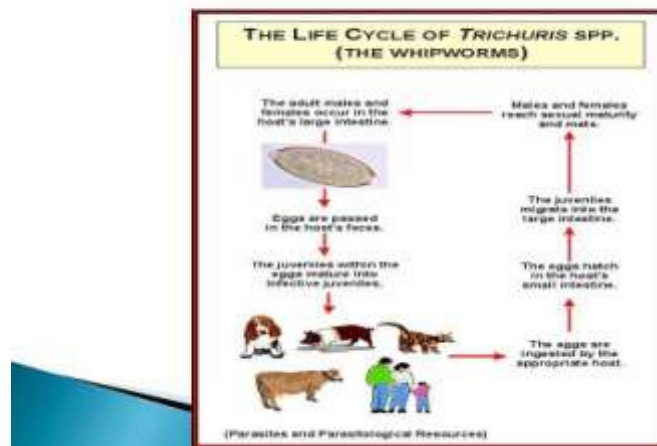


Fig:4 (Lifecycle of Whipworm)

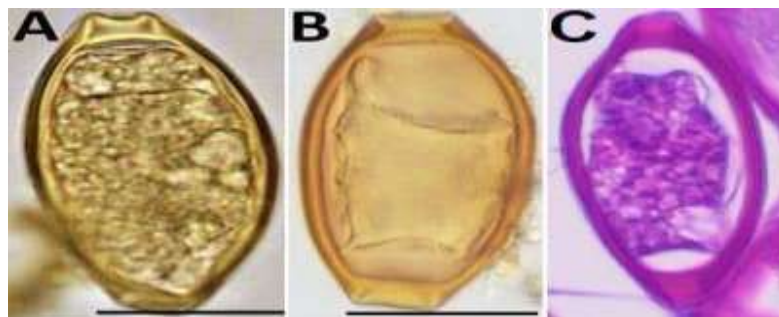


Fig:5 (Eggs of Whipworms)

4. PATHOPHYSIOLOGY

Direct damage:

Direct damage is done by worm activity itself, such as internal organ blockage or direct pressure effects by growing parasites.

- Adult *Ascaris* blocks the intestine that leads to small bowel obstruction, volvulus, or intussusception, especially in children, or can invade orifices leading to appendicitis, cholecystitis, pancreatitis, and gastric ascariasis. Migrating *Ascaris* can also block the bile duct and may also alter the intestinal microbiota. Mucosal bleeding from the upper gastrointestinal tract or generalized inflammation leads to anemia.
- *Trichuris* lies in intestinal mucosa and can cause petechial lesions, blotchy mucosal hemorrhage, oozing, and colonic inflammation. It can also cause severe anemia in pregnant women.
- Schistosomiasis infection is acquired by contact with contaminated freshwater, especially during swimming or washing. Deposition of schistosome eggs within the liver and bladder may form granulomas around these eggs that can block blood flow in the liver that leads to pathological changes like periportal fibrosis and have been linked with neoplasia. Interestingly, this periportal fibrosis has retained hepatocellular function that is different from other causes of cirrhosis. These liver flukes can also cause bile duct hyperplasia.
- *Wuchereria bancrofti* causes lymphatic obstruction leading to elephantiasis. Hydatid cyst caused by the larval tapeworm infections (*Echinococcus granulosus*) leads to pressure atrophy.
- *Taenia solium*, the pork tapeworm, frequently develops in the intestine leads to taeniasis, and in the central nervous system leads to cysticercosis.
- *Ancylostoma* and *Necator* burrow their teeth into mucosa and submucosa, create negative pressure by contracting their muscular esophagi that lead to rupture of the capillaries and arterioles and actively suck blood. Blood vessels are ruptured by both mechanical compressions and hydrolytic enzymes secreted by these hookworms. These worms also secrete anticoagulants that lead to prolonged bleeding and, ultimately, significant blood loss. They can cause significant anemia, especially in children and pregnant women, along with schistosomiasis, these can increase neonatal prem

aturity and maternal morbidity and mortality, also causes protein loss by inflammation.

- *Diphyllobothrium latum* causes vitamin B12 deficiency through interfering with the absorption through the intestine. Migration through body tissue, many helminths cause direct tissue damage and also by hypersensitivity reactions, whereas most affected organs are skin, lungs, liver, and intestines.

Indirect damage:

Indirect damage is done by the host immune response against the helminth.

- All helminths are antigenic to the body because they are foreign bodies and stimulate the immune response. Lymphatic blockage by *W. bancrofti* and granuloma formation by schistosomes in the liver and bladder are associated with hypersensitivity reaction against these helminths.
- *Strongyloides* and *Trichinella* may induce prolonged inflammation of the intestine that causes villous atrophy; in severe cases, it may cause protein-losing enteropathy.
- *Stercoralis* can cause Loeffler syndrome by type I hypersensitivity reaction.
- *Trichuris*, which is also known as whipworm, can cause inflammation of the colon that leads to blood loss and rectal prolapse.

SIGNS & SYMPTOMS

- Abdominal pain
- Nausea
- Loss of appetite
- Weight loss
- Cough
- Visible worms in stools (in some cases)

Hookworms

- Skin rash on the feet where the larva entered the body
- Fever
- Coughing or wheezing
- Abdominal pain
- Loss of appetite
- Diarrhea
- Weight loss
- Anaemia

Roundworms

Early-phase symptoms;

- High temperature fever of 38°C (100.4°F) or above

- Adrycough
- Shortness ofbreath
- Wheezing

Late-phasesymptoms;

- Passingaworminyourfeces
- Mildabdominalpain
- Nausea
- Vomiting
- Diarrhea

Whipworms

- Bloodydiarrhea
- Abdominalpain
- Painfulorfrequentdefection
- Nausea
- Headaches
- Suddenand unexpected weightloss
- Fecalincontinence,ortheinabilitytocontroldefecation

COMPLICATIONS

Alotofcomplications canoccur inhelminthinfection, whichmayinclude:

- Anemia
- Malnutrition
- Growth retardation
- Developmentalretardation
- Intestinalobstruction
- Gastrointestinalhemorrhage
- Corpulmonale
- Portalhypertension
- Urinarybladder carcinoma
- Neurologicalcomplicationssuchasseizure,myelopathy

- Primaryand secondary infertility
- Ectopicpregnancyandtubalpregnancy
- Hypogonadism
- Systemiccysticercosis
- Cholangitis
- Cholecystitis
- Pancreatitis
- CystorHydatidcystrupture
- ChroniclymphaticdamageBlindness

DIAGNOSIS

Themost commondiagnostictestsforhelminthiasis include:

- Stooltest
- Bloodtest
- Tape test
- Colonoscopy/Endoscopy
- X-ray,MRIscan,CTscan

TREATMENT:

Proper hygiene maintenance is one of the most important measures to prevent helminthinfection.ForthetreatmentofA.lumbricoide s,severaldrugsmaybeused,including albendazole,mebendazole,pyrantelpamoate,levamisole,andivermectin.Ifpatientsdevelop intestinal obstruction, it requires propertreatment withintravenous support,anthelmintics, andantibiotictreatment.Laparotomy mightbenecessa ryincaseofsmallbowelobstruction, intussusception, and volvulus.Hepatobiliary ascariasis can be treated with drug therapy. If conservative therapy fails, then endoscopic and surgical therapy may be required.

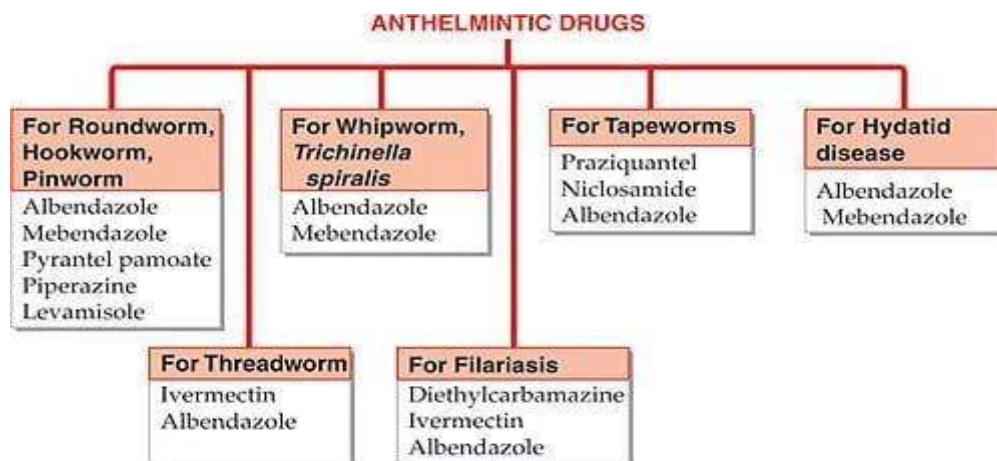


Fig:6(classificationofanthelminticdrugs)

STANDARD DRUG PROFILE
ALBENDAZOLE

Albendazole is an anthelmintic (anti-worm) medication. It prevents newly hatched insect larvae (worms) from growing or multiplying in your body. Albendazole is used to treat certain infections caused by worms such as pork tapeworm and dog tapeworm. Albendazole may also be used for purposes not listed in this medication guide.



Fig: 7 (Albendazole)

Mechanism of action:

Albendazole causes degenerative alterations in the tegument and intestinal cells of the worm by diminishing its energy production, ultimately leading to immobilization and death of the parasite. It works by binding to the colchicine-sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules. As cytoplasmic microtubules are critical in promoting glucose uptake in larval and adult stages of the susceptible parasites, the glycogen stores of the parasites are depleted. Degenerative changes in the endoplasmic reticulum, the mitochondria of the germinallayer, and the subsequent release of lysosomes result in decreased production of adenosine triphosphate (ATP), which is the energy required for the survival of the helminth.

PHARMACOKINETICS:

Absorption: Poorly absorbed from the gastrointestinal tract due to its low aqueous solubility. Oral bioavailability appears to be enhanced when co-administered with a fatty meal.

Distribution: 70% bound to plasma protein and is widely distributed throughout the body.

Metabolism: Liver **Elimination:** Urine **Half-life:** 8-12 hrs

Side effects:

- Stomach pain
- Nausea

- Vomiting
- Headache
- Dizziness
- Reversible hair loss
- Peeling skin
- Swelling of the mouth, face, lips, tongue
- Rashes

Uses:

- For the treatment of parasitic worm.
- This is used for the treatment of cysticercosis and disease of the liver and lung caused by dog tapeworm.

PLANT PROFILE

The Lemon is a species of small evergreen tree in the flowering plant family of Rutaceae, native to Asia, primarily North East India (Assam), Northern Myanmar and China.



Synonym: Citrus Limon

Habitat: Lemon trees are sub-tropical plants native to Asia. This evergreen produces fragrant flowers, has dark green leaves and bears nutritious, edible fruit. **Fig: 8 (lemon plant)**

Lemon trees require less heat than other varieties of citrus. Outstanding varieties for the home garden include "Improved Meyer" with very juicy fruit and "Variegated Pink Eureka" with fruit featuring green and yellow striped skin and pink flesh.

The best conditions for a lemon tree start with temperatures between 77 and 86 degrees Fahrenheit. However, growth grinds to halt at temperatures above 104 degrees Fahrenheit. Lemon trees do best when they receive at least 6 hours of sun. They prefer the warm places in your yard, typically on the southern or western side of your property, and don't grow well when exposed to cool breezes. Lemon trees prefer well-drained, sandy loam soil with soil pH between 6.0 and 7.5. They don't thrive in heavy clay soil.

DESCRIPTION:

Lemon, Citrus limon, is a small evergreen tree in the family Rutaceae grown for its edible fruit which, among other things, are used in a variety of foods and drinks. The tree has a spreading, upright growth habit, few large branches and stiff thorns. The tree possesses large, oblong or oval, light green leaves and produces purple-white flowers in clusters. The lemon fruit is an ellipsoid berry surrounded by a green rind, which ripens to yellow, protecting soft yellow segmented pulp. Lemon trees can reach 3–6m (10–20ft) in height and can live for many years, reaching full fruit bearing capacity in approximately 40 years. Lemon may also be referred to as bush lemon or Persian apple and likely originated from the eastern Himalaya of India.

Stem: the lemon plant stem forms an evergreen spreading bush or small tree, 3-6 meters (10-20 feet) high if not pruned.

Leaves: lemon leaves are small to medium in size and are ovate, oblong, tapering to a point on the non-stem end. The vibrant green leaves grow alternately along the branches, and they have fine-toothed edges with a slight rippling.

Flower: The mildly fragrant flowers may be solitary or there may be 2 or more clustered in the leaf axils. Buds are reddish; the opened flowers have 4 or 5 petals 3/4 in long, white on the upper surface (inside), purplish beneath (outside), and 20-40 more or less united stamens with yellow anthers.

Fruit: Lemon (Citrus limon (L.) Burm.) a yellow or pale yellow prolate fruit with five to 10 seeds, botanically a berry, is known throughout the world, and is used in numerous foodways and cuisines.

Calyx: Calyx cupular with 4 or 5 lobes. Petals linear-oblong, 1.5–2 cm long, white, pink abaxially. Stamens 20–40, basally coherent in bundles. Ovary subcylindric or barrel-shaped, 8–10 (11)-locular.

TAXONOMICAL CLASSIFICATION

Table:1 (Taxonomical classification)

KINGDOM	Plantae
DIVISION	Magnoliophyta
CLASS	Mangoliopsida
SUBCLASS	Rosidae
ORDER	Sapindales

FAMILY	Rutaceae
SUBCLASS	Aurantioideae
TRIBE	Citreae
GENUS	Citrus

VERNACULAR NAMES

Table:2 (vernacular names)

ENGLISH	Lemon, citrus limon
HINDI	Nimbu
TELUGU	Nimmakaya
KANNADA	Nimbe

MALAYALAM	Naranga
MARATHI	Limbu
SANSKRIT	Jambeer
TAMIL	Eleumiccai

ECONOMICAL:

Lemons are produced in large numbers in many countries worldwide. Some very large producers of lemons are China and India. Produced in negligible amounts, lemon oil production is dominated by Argentina, Spain, Italy, the USA, and South Africa. Argentina and Spain dominate world production with approximately 70% of global output.

NUTRITION:

Lemons contain very little fat and protein. They consist mainly of water (88-89%) and carbohydrates (10%). A medium lemon provides only about 20 calories. The nutrients in 1/2 cup (100gms) of raw peeled lemons are:

- Calories: 29
- Water: 89%
- Protein: 1.1 gm
- Sugar: 2.5 gm
- Fiber: 2.8 gm
- Fat: 0.3 gm

Carbs: The carbohydrates in lemons are primarily composed of fibers and simple sugars, such as glucose, fructose, and sucrose.

ZFiber:The main fiber in lemons is pectin.Soluble fibers like pectin can lower blood sugar levels byslowingdownthedigestionofsugar and starch.Dietary fibresareanimportant part of a healthydiet and linked to numerous healthy diet and linked numerous helath benfit.

VITAMINSANDMINERALS:

VitaminCAnessentialvitaminandantioxidant, vitamincisimportantforimmune function and skin health .

Potassium

A diet highinpotassiumcanlowerbloodpressurelevelsandhave positive effects on heart health .

VitaminB6.Agrouppofrelatedvitamins,B6isinvolved in converting food into energy.

Chemical constituents

The most important group of bio active compounds in citrus lemon fruit is flavonoidssuch as

:flavonones,eriodictyol,hesperidin,hesperitin,naringin;flavones-apigenin,diosmin

Flavonols-

quercetin;andtheirderivatives.Inthewholeplantother flavonoidsdetected

;flavonols-limocitrinandspinacetin,andflavones-orientinandvitexin.Someflavonoids,such as neohesperidin ,naringin hesperidin are characteristics citrus lemon plant . Lemon seeds contain 34.92% oil, constituted by major fatty acids including 21.03% palmitic acid, 3.67% stearic acid, 20.80% oleic acid, 44.31% linoleic acid, and 8.96% linolenic acid with 125.01 mg/kg total tocopherols, 4.36 mg/kg carotenoids and 1196.71 mg gallic acid equivalents (GAE)/kg total phenolics.

Industrial applications

Lemon is a highly valued citrus fruit for its multiple applications in the food, cosmetic and pharmaceutical industries. In addition, the by-products derived from it and considered food waste, such as leather, can be revalued through upcycling thanks to its different properties of interest in the agri-food industry that we will see in detail.

- Lemon Verna, which is grown mainly in Spain. Medium to large in size, it has a thick, rough and irregularly sized rind, with a tender pulp and juice with just the right acidity.
- Fine Lemon, spherical in shape, smooth and thin rind. Its pulp is juicy, with a high content of juice and few seeds. It is known by the name of Primoflor.

- Interdonato Lemon, with a large, elongated fruit, with a smooth, thin rind, it has little juice and few seeds in its pulp.
- Lemon Eureka, with a thin and smooth rind, its juice is very acidic and it has almost no seeds. It occurs in the California area although the seeds come from Italy .

Some of the properties

of lemon as a functional ingredient are:

- High amount of vitamin C and citric acid
- Carbohydrates naturally
- Source of minerals such as calcium and iron
- A very low caloric intake, 29 kcal/100 grams
- Lemon is a fruit with high amounts of vitamin C, citric acid and antioxidant activity, widely consumed globally.
- Lemon peel can be recovered, revalued through upcycling and used as a powder for various applications in the agri-food industry.
- At **Agrosingularity** we work with lemon peel obtaining a powder of the highest quality, treated to be a part of four PreNat products for its distribution and application in the agri-food industry.

PHARMACOLOGICAL ACTIONS:

Anti-bacterial activity:

There were several studies that determine the anti-microbial property in the citrus plant. Among them there were few studies that belong to anti-bacterial activity on leaves of Citrus limon plant. In one of the studies showed that increasing the essential oil concentration, increases the zone of inhibition, so that the highest antibacterial activity was noted at

10 mg/ml of essential oil. The results were analyzed by disc diffusion method and it showed essential oil exhibited maximum zone inhibition against Gram positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus faecium*) whereas the minimum zone inhibition was shown by Gram negative bacteria (*Salmonella typhi*, *Shigella dysenteriae*) at the same concentration. Because of this reason mostly essential oil rich compounds can be used as preservatives.

Evaluation of antibacterial activities of three main citrus plant leaves (*Citrus limon*, *Citrus grandis* and *Citrus reticulata*) against pathogenic bacteria has been done in 2016, that results determined Citrus limon leaf essential oil was having a greater length that act against between Gram Positive Bacteria .

Antifungal activity:

Anti-fungal activity of the essential oil that extract from Citrus limon leaves was tested by poisoned food technique and the volatile activity assay against five plant pathogenic fungi (*Alternaria alternata*, *Rhizoctonia solani*, *Curvularia lunata*, *Fusarium oxysporum* and *Helminthosporium oryzae*). Results manifested better activity in volatile activity assay. The minimum inhibitory concentration for *Alternaria alternata*, *Rhizoctonia solani* and *Curvularia lunata* was 0.2ml/100ml whereas >0.2ml/100ml for *Fusarium oxysporum* and *Helminthosporium oryzae* in poisoned food technique. Fungal sporulation was completely vanished at 2ml/100ml of the essential oil except *Curvularia lunata* and *Helminthosporium oryzae*.

Sedative, Anxiolytic and antidepressant effect:

There was a most popular Brazilian traditional herbal compound that greatly worked as sedative, anxiolytic and antidepressant. It was consisting with Citrus limon essential oils. Some studies evidenced sedative and anxiolytic effects of essential oil that might involve an action on benzodiazepine-type receptors, and also an antidepressant effect where noradrenergic and serotonergic mechanisms will probably play a role. Leaves were placed in such a way that the edge of the petiole was in contact with the bottom of a glass bottle, soaked with 0.2mM jasmonic acid and salicylic acid aqueous solutions and incubated at 25°C for 24 hours. The content of amino acids such as, tyrosine, tryptophan, phenylalanine, valine, leucine, isoleucine, lysine, methionine, threonine, histidine and γ -aminobutyric acid, was increased after this stress treatment.

Antioxidant activity:

Few studies were carried to determine the antioxidant activity of essential oils of Citrus limon, in one of the studies was arranged to study the potency of antioxidant with using rats, that revealed Essential oil of Citrus limon treatment significantly reduced the lipid peroxidation level and nitrite content but increased the glutathione reduced concentration (GSH) levels and the superoxide dismutase, catalase, and GPx activities in mice hippocampus. The antioxidant performance of citrus essential oil could be attributed to their phenolic contents found in leaves

Antinociceptive effect:

Both antioxidant and antinociceptive activities of Citrus limon leaves were examined using mice or in vitro tests. At the study when orally leaves essential oil significantly reduced the number of writhes and at highest doses, it reduced the number of paw licks. So results were supported to exhibit that the essential oil of Citrus limon acts as an antioxidant and Antinociceptive effect.

Anti-cancer effect:

In one study reported the potential of citrus limonoids as an anticancer agent in mice, it was found that five limonoids aglycones (limonin, nomilin, obacunone, isoobacunonic acid, ichangin) induced significant amounts of Glutathione-S-transferase in the liver and intestinal mucosa.

Glutathione-S-transferase is a major detoxifying enzyme system which catalyzes the conjugation of glutathione with many potentially carcinogenic compounds which are highly electrophilic in nature. A study of the inhibitory effect of two limonoid aglycones (limonin and nomilin) on the formation of benzo-a-pyrene induced neoplasia in the fore stomach of mice showed that incidence of tumors could be reduced by more than 50% at 10mg/dose.

PLAN OF WORK:

AIM:

The present study aims to evaluate the anti-helminthic activity of the ethanolic extract of Citrus Limon seeds against *Phereutima posthuma*.

OBJECTIVES:

- To collect seeds of lemon plant
- To prepare methanolic extract of seeds of Citrus limon (family Rutaceae)
- Qualitative phytochemical analysis of methanolic extract of seeds of lemon
- Anti-helminthic activity of seeds of lemon

II. LITERATURE REVIEW

- **Marta Klimek and Halina Ekiert 2020 et al** - This review presents important botanical, chemical and pharmacological characteristics of Citrus limon (lemon)—a species with valuable pharmaceutical, cosmetic and culinary (healthy food) properties. A short description of the genus Citrus is followed by information on the chemical composition, metabolomic

studies and biological activities of the main raw materials obtained from *C. limon* (fruit extract, juice, essential oil). Recently scientifically proven therapeutic activities of *C. limon* include anti-inflammatory, antimicrobial, anticancer and antiparasitic activities. The review pays particular attention, with references to published scientific research, to the use of *C. limon* in the food industry and cosmetology. Lastly, the review emphasizes the significance of biotechnological studies on *C. limon*.

- **Ramazan Sevik 2022 et al** -In this study, the composition of essential oils (EOs) obtained from *Citrus limon* L. and *Citrus sinensis* L. peels and their biological activities on foodborne pathogenic bacteria and food-borne saprophytic yeasts and molds were investigated. In the lemon peel EO, 17 components were identified, mainly limonene (68.65 %) and γ -terpinene (10.81%). Similarly, 8 components were determined in the orange peel EO, mainly limonene (95.51 %) and β -myrcene (1.98 %). The lemon and orange peel EOs showed a higher antibacterial effect on gram-positive bacteria used in the study compared to gram-negative bacteria.
- **Anis Ben Hsouna and Nihed Ben Halima 2017 et al** -In this study, we assessed chemical composition, antioxidant and antimicrobial activities of *C. limon* essential oil (CIEO) with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. Gas chromatography/mass spectrometry (GC-MS) was used to identify the major components of the obtained CIEO. The antioxidant activities of this CIEO were determined according to the β -carotene bleaching assay, as well as by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. For antimicrobial activity, agar well diffusion method was used and the minimum inhibitory concentrations (MICs) as well as the minimum fungicidal concentrations (MFCs) were determined. The in situ effect of the CIEO was evaluated through physicochemical parameters (pH and thiobarbituric acid reactive substances (TBARS)), as well as against *L. monocytogenes* in minced beef meat model.
- **Mansour Amin and Nasrin Aghel 2016 et al** -The purpose of this study was to evaluate the

antimicrobial activity of essential oil and extract of Lemon (*Citrus limon*), Mandarin (*Citrus reticulata*), and Pummelo (*Citrus grandis*) against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Salmonella typhi*. Microorganisms resistant to most antibiotics are rapidly spreading, and there is an urgent and continuous need for novel antimicrobial compounds. The genus *Citrus* belongs to the family Rutaceae and has many biologically active secondary metabolites. In this study, minimum inhibitory concentrations (MICs) of different *Citrus* leaf extracts were determined against all four foodborne pathogens. The *C. grandis* leaf essential oil had potent antimicrobial activity against all four pathogens, and the *C. limon* leaf essential oil was effective on Gram positive bacteria. *Salmonella typhi* was resistant against these two leaves' essential oils.

- **Chunlian li and Weicheng Zhang 2022 et al** -Species of the genus *Citrus* are cultivated in many regions of China and are widely used for medicinal purposes. In the present study, essential oils (EOs) were extracted from four different *Citrus* species using steam distillation. The chemical components of these four essential oils were separated using gas chromatography-mass spectrometry, and 52 compounds were confirmed. D-limonene was found to be the most abundant compound. All four essential oils demonstrated varied but remarkable radical scavenging capacity (IC₅₀; 0.77–13.9%). *Citrus paradisi* essential oil exhibited excellent antioxidant activity. Compared to ibuprofen, topical application of the four *Citrus* spp. essential oils significantly inhibited ear edema formation in mice. Furthermore, essential oils from the four *Citrus* species reduced the expression levels of interleukin-6 (IL-6), cyclooxygenase-2 (COX-2) and nuclear transcription factor κ B p65 (NF- κ B) to different degrees. These results suggest that the four *Citrus* essential oils have potential for use as active ingredients in functional foods or cosmeceutical products.
- **Mohamed makni and Hamadifetouiet 2018 et al** -Natural plant extracts contain a variety of phenolic compounds which are assigned various biological activities. Our work aims to make a quantitative and qualitative characterization of the Zest (ZL) and the Flesh

(FL) of lemon (Citrus limon), to valorize the pharmacological uses of lemon, by evaluating in vitro activities (DPPH, free radical scavenging and reducing power). The antibacterial, antifungal, and antiproliferative activities were sought in the ability of Citrus limon extracts to protect DNA and protein. We found that the ZL contains high amounts of phenolics responsible for the important antioxidant properties of the extract. These preliminary results showed that Citrus limon has antibacterial and antioxidant activity in vitro. It would be interesting to conduct further studies to evaluate the in vivo potential in an animal model.

- **Sarah zahr and Rana EL Hajj 2023 et al-** Citrus is among the main tree crops cultivated worldwide, with orange and lemon being well-known species. In this review, a thorough investigation of the literature was conducted to compare the phytochemistry of sweet orange and lemon fruits and reveal their nutritional and health implications. This review gives a thorough and critical evaluation of the composition and traditional medical uses of Citrus sinensis and Citrus limon with their pertinent bioactivity. Oranges and lemons are considered potential sources of antioxidant agents scavenging free radicals and preventing their degenerative effects because of polyphenols, flavonoids, limonoids, carotenoids, and vitamin C. Additionally, different parts of oranges and lemons have shown efficiency against various pathogenic bacteria, fungi, and cancer.
- **Shefalee k. Bhavsar and Mamta B. Shah 2007 et al** -The current study was designed to investigate the effect of Citrus limon. (L.) Burm. (Rutaceae) fruits, commonly known as lemon, in experimental liver damage. The ethanolic extract of Citrus limon. fruits was evaluated for its effects on experimental liver damage induced by carbon tetrachloride, and the ethyl acetate soluble fraction of the extract was evaluated on Hep G2 cell line. The ethanolic extract normalized the levels of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), and total and direct bilirubin, which were altered due to carbon tetrachloride intoxication in rats. The results from the current investigation also indicate good correlation between the in vivo. and in vitro. studies.
- **Chistiane Mendes Feitosa and Lidianne Mayra Lopes Campelo 2011 et al** This study investigated the effects of C. limon essential oil (EO) on lipid peroxidation level, nitrite content, glutathione reduced (GSH) concentration, and antioxidant enzymes [superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx)] activities in mice hippocampus. EO of limon treatment significantly reduced the lipid peroxidation level and nitrite content but increased the GSH levels and the SOD, catalase, and GPx activities in mice hippocampus.
- **M.H. Moosavy and P. Hassanzadeh 2017 et al-** Citrus fruits have some antioxidant and antimicrobial properties. The aim of this study was to determine the chemical compounds, antioxidant, and antimicrobial activities of Essential Oil (EO) of lemon (Citrus limon) peel in vitro and in a food model. The total phenol content was 81.82 ± 8.02 mg gallic acid equivalent/g of the EO. Also, the total amount of flavonoids in the EO of lemon peel was 11.72 ± 1.82 mg/g rutin equivalent. Lemon peel EO showed 55.09% inhibition of DPPH, showing significant difference with control group ($p < 0.05$). The MIC and MBC value of EO against *S. aureus* was 1.25 and 5%, respectively, having significant difference ($p < 0.05$) with control group. A dose-dependent manner was seen in food model revealed significantly lower ($p < 0.05$) bacterial number in EO containing barley soup groups than the control one.
- **Junab Ali and Trideep saikia 2017 et al** evaluate the antimicrobial activity of methanolic extract from the peel of the fruit of Citrus Limon (Family-Rutaceae) in conjugation with phytochemical analysis. The methanolic extract from the peel of the fruit of Citrus Limon (Family-Rutaceae) was separated from fruits, shade dried, powdered and extracted using methanol, analysed for phytochemical constituent using standard methods. The antimicrobial activity of the plant extract was examined against 2 bacterial strains among one is Gram-positive (*Staphylococcus aureus*) and other is Gram-negative (*Escherichia coli*) and 1 fungal strains (*Candida albicans*) using agar well

diffusion method. Results: Various phytochemical analyses revealed the presence of alkaloids, saponin, flavonoids, carbohydrates, glycosides and citric acids and tannins. The antimicrobial activity of the methanolic extract of the plant showed significant result against all the of the test organisms.

- Amit Pandey and Sudeep Kumar Tiwari 2011 et** The present study was carried out to find out the antimicrobial activity of ethanolic, methanolic, ethyl acetate & hot water extract of lemon fruit parts like peels & seeds. Antimicrobial analysis was done by using agar well diffusion method against bacterial and fungal pathogens. Methanolic extract of lemon peel exhibited the maximum zone of inhibition against *Pseudomonas aeruginosa* whereas hot water extract of lemon peel exhibited least zone of inhibition. Ethanolic extract of lemon seeds showed maximum zone of inhibition against *Pseudomonas aeruginosa* whereas hot water extract showed least zone of inhibition. MIC value was determined by using micro broth dilution method. The least concentration was obtained 2.4 mg/ml for ethanolic and hot water extract of lemon peels against

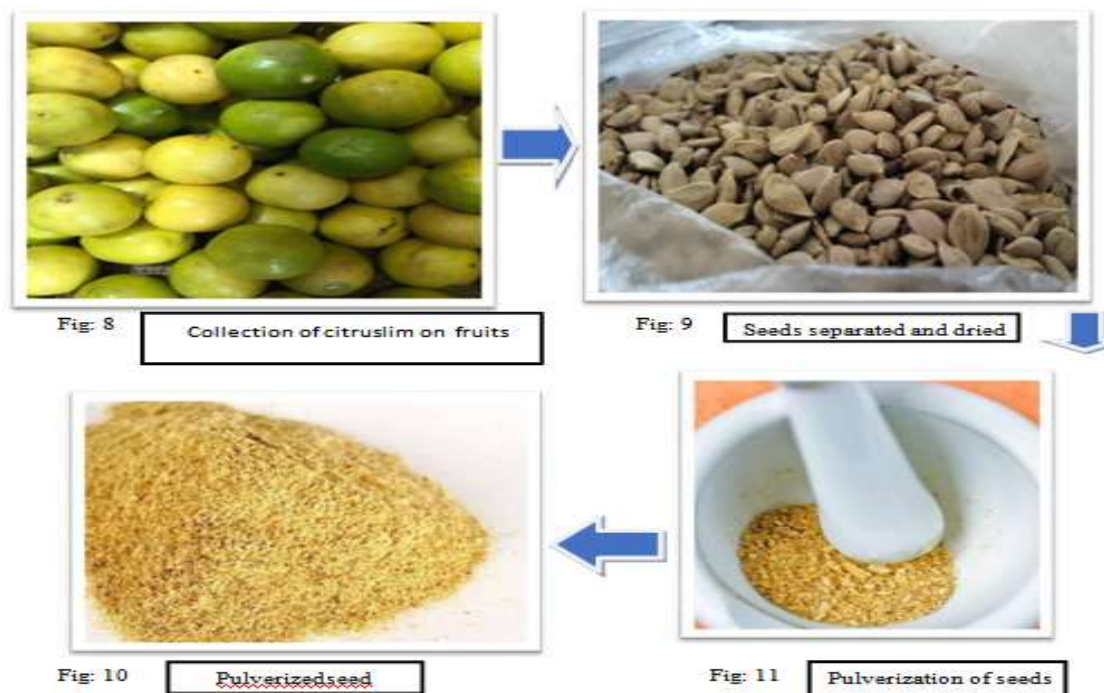
S. aureus. The MBC value also determined and phytochemical analysis showed the presence of tannins, glycosides, reducing sugars and flavonoids.



III. MATERIALS & METHODS

COLLECTION OF PLANT MATERIAL

Fresh lemon fruits were collected from the local market. The fruits were washed with sterile distilled water. After that seeds were separated from the fruits, dried under the shade at room temperature for one week, and pulverized to a fine powder using grinders.



COLLECTION OF EARTH WORMS

The Indian earthworm *Pheretima posthumus* were collected from a Vermicompost unit at pedda tekur village near kurnool, Andhra Pradesh.



Fig:12(Earthworms)

EXTRACTION BY MACERATION

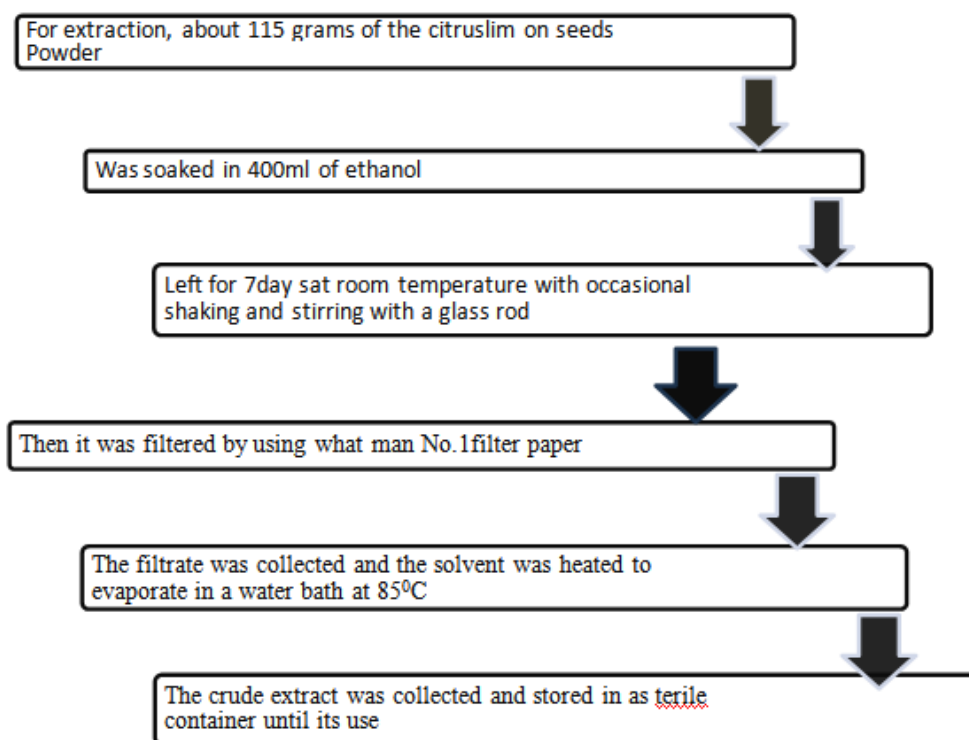




Fig:13(Maceration)



Fig:14(Filtration)



Fig:15(Filtrate)

PRELIMINARY PHYTOCHEMICAL SCREENING

The plant extract was assessed for the existence of the phytochemical analysis by using the following standard methods

Test for Flavonoids:

Shinoda test: Pieces of magnesium ribbon and concentrated HCl were mixed with lemon seed extract after a few minutes reddish color showed the presence of flavonoids.

Alkaline reagent test: 2ml of 2% w/v NaOH solution was mixed with lemon seed extract, concentrated yellow color was produced, which became colorless when it was diluted into the mixture. This result showed the presence of flavonoids.

Test for Phenol:

To 1ml of extract, 3ml of 10% w/v lead acetate solution was added. A bulky precipitate indicates the presence of phenolic compounds.

Test for Saponins:

5ml of water was mixed with lemon seed extract in a test tube and it was mixed vigorously. The frothing was mixed with a few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.

Test for Tannins:

10ml of bromine water was added to the 0.5g of extract. Discoloration of bromine water showed the presence of tannins.

Test for Carbohydrates:

Molisch's test: 2ml of extract taken in a test tube. Now two drops of Molisch's reagent are added to the extract and mix it. Now, add slowly concentrated sulphuric acid in sloping the test tube by a slide without mixing vigorously. A purple ring appears at the interface between the acid and test layers which confirms the presence of carbohydrates.

ANTHELMINTHIC ACTIVITY

Preparation of plant extract: The extract was dissolved in saline to prepare sample solution of concentrations of 25mg/ml, 50mg/ml to be used for screening the anthelmintic activity.

Standard drug: Albendazole (25mg/ml, 50mg/ml) in saline was taken as a standard solution.

Activity: The anthelmintic activity was evaluated on adult Indian earthworms. For preliminary evaluation of anthelmintic activity test samples of the extract were prepared at the concentrations of 25mg/ml and 50mg/ml. 20

worms 5-7cm were divided into 4 groups(1 control, 2standardsand2tests) eachcontaining4wormsandplaced inpetridishescontaining

the above test solutions of extracts. Albendazole was used as the reference standard and a normal

saline is used as a control.All the tests, control and standard solutions were prepared freshly before starting the experiment. Observation is made for the time taken for paralysis whenmovementwaslostornomovement.The timefort hedeathofwormswasrecordedafter ascertaining those worms neither moved in lukeworm water.

IV. RESULTSANDDISCUSSION

PHYTOCHEMICALSCREENING

Table:3(Qualitativechemical examinationofextractofcitruslimonseeds)

Nameofphytoconstituent	Ethanoliceextract
Flavonoids	+
Phenoliccompounds	+
Saponins	+
Tannins	+
Carbohydrates	+

Preliminaryphytochemicalscreeningoftheethanolice xtractofcitruslimon seedsreveals the presence of flavonoids, tannins, saponins, carbohydrates,phenolic compounds.

For monitoring anthelmintic activity different concentrations of ethanolic extract(25mg/ml,50mg/ml)weretaken.Whilenormal salineandalbendazole (25mg/ml, 50mg/ml) act as control and standard drug respectively. Time of paralysisanddeathwasobserved.

ANTHELMINTICACTIVITY



Fig.16(control)

Control group (Saline)

Standard drug (25mg/ml and 50mg/ml)



Fig. 17 (standard drug)

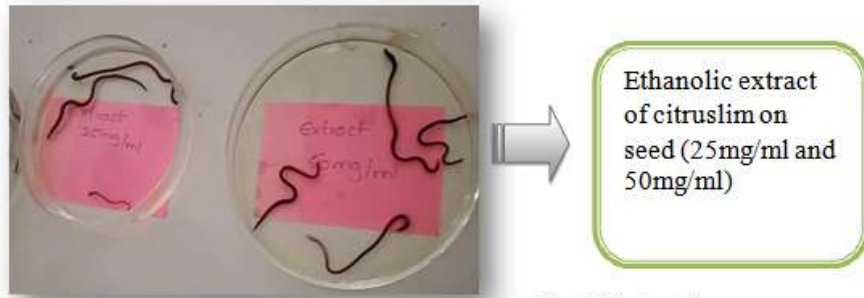


Fig: 18 (extract)

Different concentrations of ethanolic extract, control and standard drug.

Table:4 Anthelmintic activity of test (extract of citrus Limon seeds)

S.NO	CONCENTRATIONS	TIME OF PARALYSIS (MIN)	TIME OF DEATH (MIN)
1.	25mg/ml	131	156
2.	50mg/ml	28	41

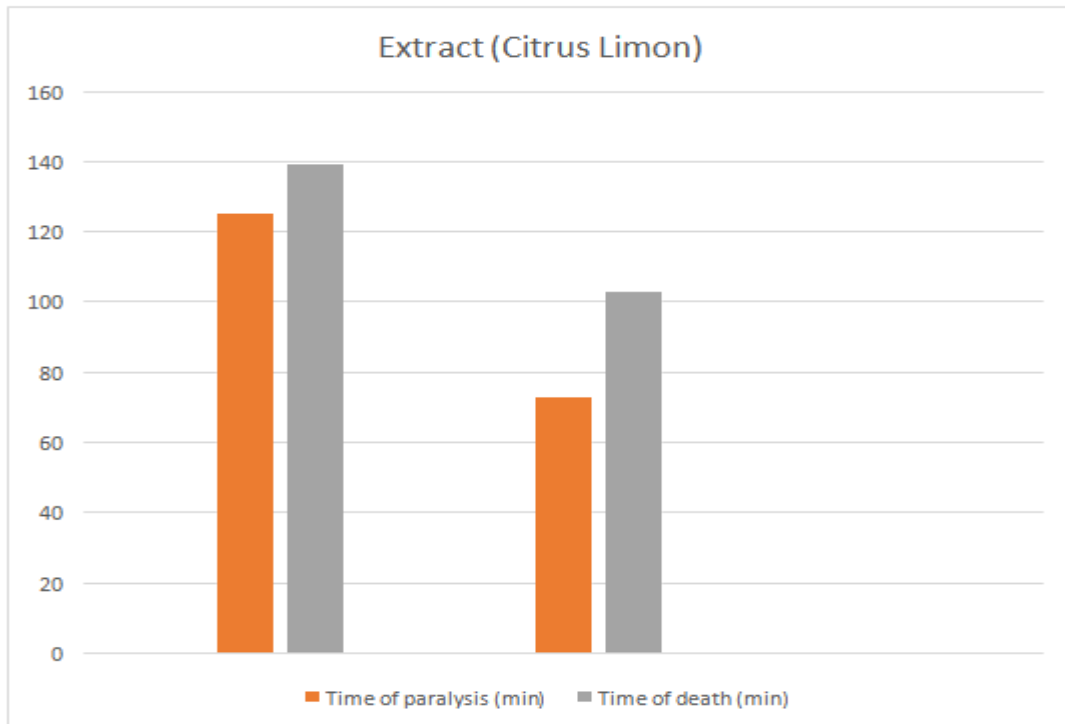


Fig:19(Anthelmintic activity of extract citrus limon seeds)

Table 5: Anthelmintic activity of standard Drug (Albendazole)

S.NO	CONCENTRATIONS	TIME OF PARALYSIS (MIN)	TIME OF DEATH (MIN)
1.	25mg/ml	125	139
2.	50mg/ml	73	103

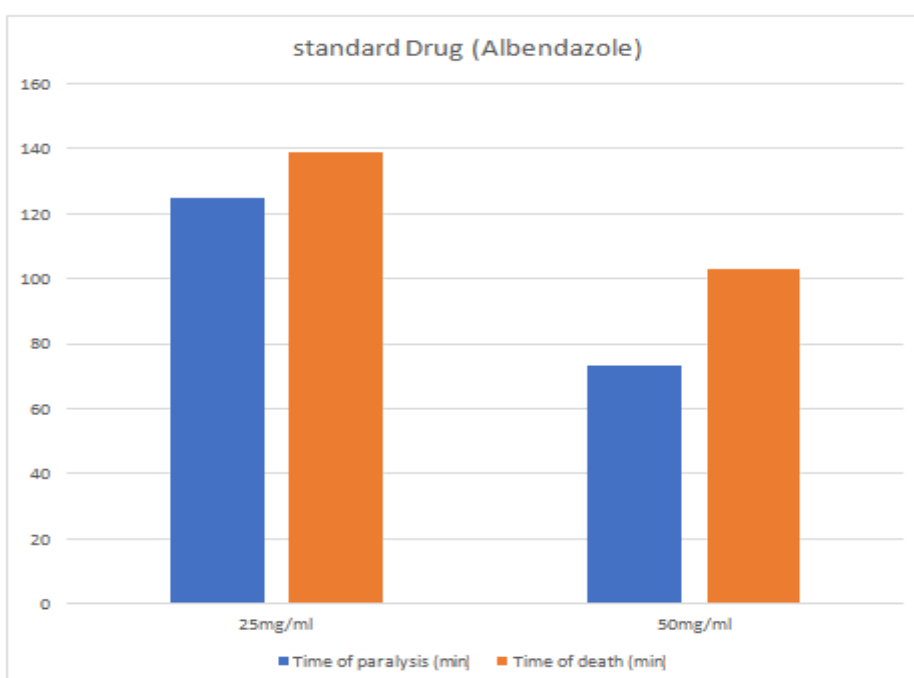


Fig: 20(Anthelmintic activity of standard Drug Albendazole)

V. DISCUSSION

Citrus Limon fruits were collected from a nearby market to prove the anthelmintic activity of the ethanolic extract of seeds. The seeds were dried, pulverized into a fine powder, and extracted by maceration with ethanol. The extract was screened for phytochemicals. The screening showed positive for flavonoids, saponins, tannins, carbohydrates and phenolic compounds. The extract was employed to determine anthelmintic activity against *Pheretima posthuma*. The results revealed a dose-dependent increase in activity of the extracts at 25mg/ml and 50 mg/ml concentration. The extracts at 50 mg/ml exhibited better activity than standard compound Albendazole.

VI. CONCLUSION:

The present study concluded that the extract has shown an anthelmintic activity against Indian earth worms (*Pheretima posthuma*) due to the presence of flavonoids, phenolic compounds and tannins. At all concentrations of 25 mg/ml and 50 mg/ml, the extract exhibits anthelmintic action as determined by the duration of the worms' paralysis and eventual death. However, 50 mg/ml demonstrated the most substantial activity and was comparable to the standard drug 25 mg/ml and 50 mg/ml albendazole.

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