



COCONUT LEAVES: ANTIBACTERIAL & ANTIFUNGAL POTENTIAL

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Abstract: People have used plants as medicine from the beginning of time. Aspirin, (which is derived from willow bark), digoxin, and both morphine and quinine, (were all developed from opium poppies and cinchona bark, respectively, a century ago) which are all valuable pharmaceuticals today. The Arecaceae family includes the tropical perennial crop known as coconut (*Cocos nucifera* L.), which has a variety of applications. It is cultivated in more than 80 different countries and sustains life in fragile coastal and island ecosystems. Since every component of the coconut tree (*Cocos nucifera* L.) has value in some form, it is referred to as "Kalpavriksha". This research demonstrates the potential of coconut leaves to exhibit significant anthelmintic activity, good antibacterial and antifungal activity.

Index Terms - Coconut, *Cocos nucifera* (L), Kalpavruksha, Anthelmintic, Antibacterial and Antifungal.

I. INTRODUCTION

For a very long time, medical uses of herbal plants have been practised. The use of plant materials as a source of medicines for a wide range of human ailments has received more attention as a result of factors including population growth, insufficient drug supply, prohibitive cost of treatments, side effects of several synthetic drugs, and development of resistance to currently used drugs for infectious diseases. According to recent estimates from the World Health Organization 80% of people worldwide rely on herbal remedies for some part of their primary healthcare requirements. Around 21,000 plant species have the potential to be used as medical plants according to the WHO.

Since ancient times, people have employed plants for therapeutic purposes. A century ago, the majority of the few available medications that were actually useful such as aspirin (derived from willow bark), digoxin (from foxglove), both morphine and quinine were derived from opium poppies and cinchona bark respectively.

Coconut (*Cocos nucifera* L.) is a tropical perennial crop with several uses belonging to family Arecaceae. It supports life in vulnerable coastal and island habitats and is grown in more than 80 different nations. Since every part of the coconut tree (*Cocos nucifera* L.) is valuable in some way, it is known as "Kalpavriksha."

While playing a significant antibacterial role, antibiotics and other chemical antimicrobial agents can have a number of negative side effects. The production of free oxygen radicals is one of the primary adverse effects (ROS). ROS are extremely hazardous, and they are believed to be a major factor in the development of cancer. An antifungal drug may cause abdominal pain, upset stomach and diarrhea, Itchy skin, burning sensation or skin rash and serious problems like Liver damage (jaundice). Anthelmintic drugs may cause nausea, vomiting, diarrhea, stomach/abdominal cramps, headache, drowsiness, dizziness, trouble sleeping, loss of appetite and acute psychotic reactions. Therefore, we need to find more herbal plants which have antibacterial, antifungal and anthelmintic properties and minimum or no sideeffects and is useful to treat effectively

In a thorough examination of parasitology in the works of the ancient Greek and Latin authors, Moule (1911) evaluated the many names given to the Entozoa. parasites in the intestines Greeks employed three distinct terms: The less specific term was (theria), which was used to name intestinal worms and primarily denoted "wild animals" or, more generally, "animals": "Intestinal worms are especially abundant at autumn."

Hippocrates used the term scolex, which normally denoted insect larvae but was also used when reporting on intestinal parasites of horses: "Worms severely disturb horses and are not easily cured," Hippocrates wrote. Aristotle used the term "helminthes," which is also known as "elminthes," "elmintes," or "elmins" in French, to refer to worms that develop in animal excrement either before or after the stools have been discharged from their guts. The ascarides, flat worms, and round worms are the three different types. In particular, the human body contains these sorts.

This blatantly implies that among the several names for worms, "helminth" quickly came to denote worms dwelling in the gastrointestinal tracts of humans and other animals, becoming associated with the notion of parasitism in general. In addition, the ideas of parasitism came into play once this word was coined.

Eukaryotic parasites include parasitic worms, often known as helminths. They are worm-like creatures that feed and survive off of living hosts, obtaining nutrition and protection while interfering with their hosts' ability to absorb nutrients, leading to sickness and weakening. those that inhabit both humans and other animals. The study of parasitic worms and how they affect their hosts is known as helminthoogy. Greek helminths, a type of worm, are whence the name "helminth" originates.

The two main phyla of helminths are. The nematodes (also known as roundworms) involve the most common intestinal worm (also referred to soil-transmitted helminths) as well as the filaria worms which trigger lymphatic filariasis (LF) and onchocerciasis, while the platyheminths (also known as flatworms) contain the flukes (also referred to trematodes), like the schistosomes and the tapeworms (also referred as cestodes), such as the pork tapeworm which trigger cysticercosis.

Characteristics

- Eukaryotic, multicellular creatures that typically have reproductive, excretory, nervous, and digestive systems.
- Worms have endoderm, mesoderm, and ectoderm tissue differentiation, as well as bilateral symmetry, head and tail.

Parasitic helminths spend most or all of their lives in host and usually have the following specialization:

- A digestive system could be absent. Use the host's food, bodily fluids, or tissue to absorb nutrients.
- Possess a diminished neurological system.
- Lack of or diminished means of movement.
- An intricate reproductive system. Eggs produced by individuals can spread an infection to another host.

Acquisition

Helminthes frequently enter a host by mosquito transmission, consuming contaminated food or water, or awakening on contaminated soil. This is especially problematic in underdeveloped countries where many people lack access to shoes and where food and water are frequently unhygienic. Many people go long distances in their bare feet merely to get tainted water for their family, picking up parasites and illnesses in the process.

Immune response

Immune system effects of parasitic worms Th2 response is the most common response to worm infection in humans. There might be intestinal inflammation, which would cause cyst-like formations to develop all throughout the body around the egg deposits. The longer helminths persist, the more toxins they produce after feeding, which in turn stresses the host's lymphatic system. These poisons are discharged into the intestines where they enter the circulation of the victim. The host becomes more vulnerable to more widespread illnesses like seasonal viruses and bacterial infections as a result of this process.

Intestinal helminths

An intestinal parasite that lives in the human digestive system is called an intestinal helminth. They exhibit one of the parasite disease's most common types. According to experts, more than a quarter of the world's population has intestinal worms of some kind, including roundworm, hookworm, and whipworm. Children may also be more vulnerable to the negative effects of helminth infections because of their immature physical development and their weakened immune systems.

Distribution

There are more than 10,000 different species of parasitic flukes, according to estimates. There are over 5000 species of tapeworms. With approximately 15000 species, roundworms are an exceptionally varied category. Almost every kind of environment has roundworms, tapeworms, and flukes.

Multicellular eukaryotic organisms called helminths typically have reproductive, neurological, excretory, and digesting systems. Some people live in the water and dirt for free. Because they spread contagious diseases and can typically be identified by microscopic inspection of eggs or larvae, helminths are investigated in microbiology. Striations, spines, or opercula—the mechanism by which the larva hatches—can all be seen on eggs.

More than one-third of the world's population is infected by helminths. Helminth infections are different from bacterial and protozoan diseases in that the worms often do not multiply inside the host. The usual causes of

symptoms are mechanical harm, consuming host tissues, or vitamin deficiency. We will look at prepared slides of parasitic helminths in this practise.

Biodiversity

Three major assemblages of parasitic helminths are recognised:

- Nematelminthes (nematodes)
- Platyhelminthes (flatworm)
- Cestoda (tapeworm)
- Trematoda (flukes)

✓ **Nematodes**

Roundworms or nematodes are cylindrical in shape with tapered ends. The mouth, gut, and anus make up the whole digestive system of roundworms. Many creatures have two sexes. Long tubules that act as the ovaries or the testicles make up the reproductive system. Sperm are guided to the female's vaginal pore by one or two posteriorly located, hardened spicules in females. Spicule structure is frequently used to identify species.

✓ **Cestodes**

Intestinal parasites include cestodes and tapeworms. In microbiology, their structure is demonstrated. For attachment to the final host's intestinal mucosa, the head, or scolex (plural: scoleces), bears suckers. Some species also have tiny hooks. The tissue of their hosts is not consumed by tapeworms since they have no digestive system at all. They absorb food via their cuticle to absorb nutrients from the small intestine. As long as the scolex is connected and alive, proglottid segments, which make up the body, are continuously produced in the neck area. Male and female reproductive organs are present in every adult proglottid. As older proglottids are pushed away from the neck by younger ones, each one grows. Each proglottid has both male and female reproductive organs, and as it moves towards the centre of the worm, eggs begin to fertilise. In essence, the proglottid that is furthest from the scolex is a sack of fertilised eggs that will be expelled in faeces.

✓ **Trematodes**

Trematodes, often known as flukes, frequently feature flat, leaf-like bodies with ventral and oral suckers that serve to stabilise the animal. Flukes consume food by absorbing it via the cuticle on their outer layer. In accordance with the tissue of the definitive host in which the adult resides, flukes are given common names (for example, lung fluke, liver fluke, and blood fluke).

Helminths do not multiply within their hosts, in contrast to other diseases (viruses, bacteria, protozoa, and fungus). Worms develop, shed their skin, reach adulthood, then generate young that are ejected from the host to infect other hosts. Therefore, intake (the number of infectious stages taken in) is a factor in determining the worm loads in specific hosts (and frequently the degree of illness). Worms develop more slowly than other infectious pathogens, therefore any illnesses they cause have a chronic and slow-onset character. Subclinical infections have been linked to a considerable loss of condition in infected hosts, despite the fact that the majority of helminth infections are well tolerated by their hosts and frequently asymptomatic. Other helminths can produce severe clinical illnesses with a high morbidity and fatality rate. Depending on the location and length of the illness, the clinical indications of infection might vary greatly. Larval and adult nematodes settle,

move, or encyst inside tissues, causing blockage, swelling, anaemia, lesions, and the development of granulomas. Adult cestode infections are often benign because they are not invasive, but the larval stages can infiltrate tissues and encyst there, causing inflammation, lesions that take up space, and organ dysfunction. Blood flukes' eggs can lodge in tissues and induce widespread granulomatous responses, hypertension, and blockage in tubular organs, as opposed to the adult flukes' normal effects of inflammation, fibrosis, and obstruction.

Life Cycle of Intestinal Helminths

Among intestinal worms, *Ascaris lumbricoides* is one of the most prevalent. It is a roundworm, and the condition it causes is known as ascariasis. Horses, cattle, pigs, poultry, dogs, cats, and a variety of other animals are all impacted by species that are closely related to *Ascaris*. *Ascaris* has a life cycle that is similar to that of its relatives, which are generally referred to as ascarids. Adult ascarid worms reside in the intestines, where they lay a large number of eggs that are excreted by the host along with the waste. The eggs hatch in the small intestine of the new host if they consume food or water contaminated with these faeces.

The juvenile worms tunnel into the intestine's walls and invade nearby blood vessels. The little worms end up in the lungs after being circulated in the blood. They emerge into the airways from this point and ascend into the throat, where they are swallowed. They develop when returned to the intestines, and the cycle is again repeated.

A: Egg ingested

B: L1 small intestine

C: L2 migration

D: L3 lungs

G: Adults

- Egg consumption triggers infection [A]
- In the small intestine of the host, larvae hatch [B].
- Gut wall penetration [C]
- Migration up the trachea via the circulatory system, lungs, and [E]
- Pharyngeal movement [F]
- Maturation takes place in the upper small bowel, from whence they are ingested [G]
- The length of an adult worm can exceed 35 cm. An infection often has eight to ten worms. Eggs are excreted in faeces.

Helminthic Therapy

- *Anthelmintics*

Anthelmintics are medications that kill or stupefy helminth parasite worms (helminth) so they may be eliminated from the body. They may alternatively be referred to as vermifuges (awesome) or vermicides (deadly). However, they have demonstrated the development of resistance to some narrow-spectrum wormers like salicylanilides (closantel) and several broad-spectrum anthelmintics (benzimidazoles, levimasole, and avermectins). In many regions of the world, anthelmintic resistance is a significant barrier to the production

of cattle. There is now a constricting area in the field of study because of the frequency of parasitic diseases and the established resistance of some anthelmintic drugs.

Two billion individuals are affected by helminthiasis, a serious issue that leads to starvation, blindness, debility, disfigurement, and death. Since practically all of the native population in many countries, particularly those in tropical and subtropical regions, is infested with helminthes, their treatment is of utmost practical significance.

Schistosomiasis (blood fluke)	Antimonials, Metrifonate, Oxamanaquine, praziquantel
Cestodiasis (tape worm)	Niclosamide, Benzimidazoles, Paraziquantel
Fasciolosis (liver fluke)	Paraziquantel, Closantel and halogenated salicylamides
Intestinal round worms	Piperazine, Benzimidazoles, Morantel, Pyrantel, Levimasole, Avermectins and milbemycins, Closantel (and halogenated salicylamides), Emodepside
Filariasis (tissue round worms)	Diethyl carbamazine, Suramin, Ivermectin

Key drugs registered for the treatment of parasitic worm in humans

General Consideration for antimicrobial activity

Nearly half of all fatalities in developing and tropical nations are attributed to microbial infections. The creation of antibiotics for the treatment of infectious illnesses is one of modern medicine's greatest achievements. Sulphonamides, a synthetic antibacterial molecule, were discovered by Domagk in 1935. Penicillin was first made available to consumers in 1942, and its amazing healing powers sparked the ongoing research of more antibiotics. There have been studies on several hundred plant species with possible antibacterial capabilities. Numerous species from numerous plant groups have produced items with antibacterial characteristics. The most prevalent kinds of phytochemicals that have demonstrated potential efficacy against a variety of bacterial species include phenols and polyphenols, alkaloids, and glycosides. A significant level of antibacterial activity has also been observed in several volatile essential oils of frequently used culinary herbs and spices. The plant species included in these investigations have been selected based on their ethnomedical or traditional uses.

Antimicrobial investigations on pathogens have been conducted, and the most popular techniques used include agar, agar disc diffusion, agar broth dilution, and macro/micro broth dilution. In relatively few studies, plant extracts and phytochemicals have displayed higher and broader-spectrum efficacy compared to conventional antibiotics, and they have demonstrated more effectiveness against gram-positive pathogens than gram-negative ones. The study of phytochemicals' mechanisms of action and the conversion of in vitro research findings into in vivo, preclinical, and clinical trials both receive minimal attention. In a time when drug resistance is a threat to the entire planet, this might be a big advancement. The effectiveness obtained

utilising herbal formulations based on traditional and ethnomedical uses against a variety of bacterial illnesses encourages hope for the future of phyto-antibiotics. However, our strategy should be interdisciplinary for the future development of safe and effective antibiotics based on ethnomedicine. The development of antimicrobial drugs based on the indigenous and local knowledge of plants is a lucrative and huge unexplored source and has a great deal of potential. It is possible to look into potential antibacterial activities further.

Today, further research into therapeutic plants is required since these plants have demonstrated the existence of tiny creatures on and inside of humans since the beginning of time. They have significantly shaped human existence, according to an excerpt from the Putranjiva roxburghii Wall. Examining the Physicochemical and Pharmacological Properties of Seed Oil and Leaves. Potential pathogens are present among the microbes. The two types of microorganisms most frequently utilised are bacteria and fungus. A significant class of microbe that first arose on earth billions of years ago is the bacterium. They are the most prevalent and prolific type of life. Numerous indications suggest that many bacterial diseases are as ancient as humanity itself and have been a problem for people since the dawn of time. Bacterial infections are the leading cause of death in underdeveloped and tropical nations, thus finding a secure and highly effective treatment for microbial diseases continues to be a key problem for modern research. It wasn't until Louis Pasteur's "Germ Theory" and Koch's postulate that the link between infectious illnesses and germs was established beyond a reasonable doubt. Old and new hypotheses provided more proof that just a tiny fraction of the millions of extant microorganisms were harmful in nature. Even these, however, could only establish themselves and spread illnesses if they had overcome and weakened our immune system. Their virulence and pathogenicity are key factors in this capacity.

Present Scenario

The 21st century has witnessed the emergence of novel microorganisms with astounding virulence, the emergence of new microbial illnesses, and an alarming increase in infection-related fatalities in wealthy nations. Diphtheria outbreaks in Eastern Europe, plague outbreaks in India, and Staphylococcal infections in the West are only a few of the events that have disproved our theory that infectious diseases would be entirely eradicated by the end of the 20th century. But many of these illnesses have also been the root of long-term impairments and deformities, so this is only half the picture.

The following are some aspects that have contributed to the development of modern microbial pathogenesis:

- Emerging diseases (HIV, Hantavirus, Rotavirus, etc.) identification
- Microbiological illnesses with unclear causes
- appearance of illnesses that were previously rare
- ancient pathogens with high pathogenicity making a comeback
- diseases caused by bacteria that cannot be grown
- The connection between some diseases that were previously believed to have a physiological cause and microbial infections (such as the links between Hepatitis B infections and liver cancer and Helicobacter pylori and gastric ulcers and other types of stomach cancer)
- Opportunistic infections in older people and others with weakened immune systems

The causes of the aforementioned factors include changes in the socioeconomic environment, globalisation, and an increase in multidrug resistance among common pathogens worldwide (such as multidrug resistant tuberculosis) as a result of inappropriate and excessive use of antibiotics, noncompliance with an antibiotic prescription, and genetic differences among bacterial species. Each of these is reducing the clinical utility of antibiotics.

Plant source of Antibiotics

Plants have long been a source of several potent anti-infective substances. Even today, we discover that plant products serve as models for a variety of contemporary pharmaceuticals. The usage of higher plants for the treatment of infectious diseases predates the creation of written records. Some typical examples of traditional antibacterial medicine are the bacteriostatic and fungicidal activities of lichens, the antimicrobial activity of allicin in garlic (*Allium sativum*), or the berberines in *Hydrastis canadensis*. The screening and assessment of plant-derived antimicrobials continued even after the discovery of microbiological and synthetic antibiotics and the miraculous healing offered by penicillin and sulfa medicines in the 1940s. In reality, systematic and organised research into plant-derived antimicrobials in labs began in 1926 and continues to this day.

The extent of co-evaluation between plants and their natural enemies, such as people, animals, and microorganisms, goes well beyond what the present theories of reciprocal interaction imply. Infinite biochemical possibilities are made possible by counter-resistance, genetic flexibility, polymorphic immunological capability, and polymorphism among microbiological agents. In order to respond to these environmental dangers, plants create an enormous variety of natural substances (phytochemicals) with various antibacterial capabilities. Phytochemicals are byproducts of secondary metabolism, and among the several groups of secondary metabolites include flavonoids, alkaloids, phenols, terpenes, and resin acids. The variety of compounds is astounding since they have a wide range of chemical, physical, and biological characteristics. Even in ancient times, some of these phytochemicals were depleted and used to treat infectious disorders.

Herbal Medicines against Microorganism

Due to the complexity of current antibiotics, there has been a noticeable shift in recent years towards complementary therapies and herbal medicines. The Indian pharmaceutical industry is currently focused heavily on research and development. Antibiotics screening of plants and natural items used in alternative medical systems like Ayurvedic and Unani is a key focus. This is the rationale for the investigation and characterization of the plants and commercial herbal products used in alternative medicine. With regard to a wide range of pathogenic organisms, these studies have validated the antibacterial ability of herbal concoctions and extracts of medicinal plants used in infection control; high activity against multi-drug resistant *Salmonella typhi* has also been observed. The effectiveness of chewing sticks, whose use is so deeply ingrained in many cultures, has also been proven to be beneficial for controlling gingivitis and guarding against food-borne viruses. According to research, several herbs really promote the development of the local microflora, which in turn fights off harmful organisms and assists in the prevention and management of infectious bacteria. Numerous Indian plants, including black pepper, clove, garlic, neem, *Terminalia chebula*, tulsi, and turmeric, among others, have also been found to have substantial antibacterial action.

Investigations based on ethnopharmacological, taxonomical, and chemical information are currently being conducted all over the world. Chemotaxonomic studies on Indigenous Systems of Medicine (ISM) medicinal plants have produced a number of compounds with intriguing antibacterial activities.

- Phenolic and Polyphenolic

Phenols make up the biggest group of plant secondary metabolites that are present in most naturally occurring chemicals with aromatic moiety. They range from straightforward compounds with a single aromatic ring to extremely intricate polymeric molecules. Simple phenols, flavonoids, tannins, coumarins, anthraquinones, lignin, and their derivatives are the medicinally significant phenolics.

- Additional group of compounds:

While essential oils, vegetable oils, and their constituent parts, as well as phenolics, have all been identified in the literature as major antibacterial phytoconstituents, additional groups of chemicals have also been discovered to exhibit antimicrobial action. Alkaloids and glycosides are two groups of compounds that have a wide range of biological effects as well as substantial antibacterial properties.

- *Anti-bacterial activity*

Numerous microbes are continually present in our body. When germs enter the body, they can be so aggressive that disease is frequently the result.

Nearly half of deaths in developing and tropical nations are caused by microbial illness. The greatest advancement in therapeutics throughout the 20th century was the development of antibiotics. They are one of the few treatments available. Their significance is heightened in affluent nations where infectious diseases are more prevalent. Prontosin red dye yielded synthetic antibacterial sulfonamides, which Domagk discovered in 1935. Sulfonamides were produced in vast quantities and utilised widely. The first, least harmful antibiotic, penicillin, was found in 1941. There have been studies on several hundred plant species with possible antibacterial capabilities. Numerous species from numerous plant families have produced goods with antibacterial characteristics. The most prevalent groups of phytochemicals that have demonstrated promising efficacy against a variety of bacterial species are phenols and polyphenols, alkaloids, and glycosides. Some volatile essential oils from species and herbs that are frequently used in cooking have also demonstrated strong antibacterial properties. The plant species used in these investigations have been selected based on their traditional or ethnomedical uses.

By examining the growth response of different microbes to those plant tissues or extracts, which are placed in touch with them, it is possible to identify the antimicrobial activity of plants. Three conditions required to be met in order to find antibacterial activity in plant extracts. First, the cell wall of the pre-selected bacteria for the test was in contact with the plant extract. Second, the environment was changed to allow for microbial growth even in the absence of antimicrobial treatments. Third, there were methods for determining whether or not the test organism had grown within the time period designated for the experiment.

Antimicrobial agents are substances that either eradicate or stop the development of microbes (often known as "bugs" or "germs") such bacteria, viruses, fungi, or protozoa. Some antimicrobials are made by bacteria directly (the penicillium mould makes penicillin, for instance), whereas others are created in a lab.

- **Anti-fungal activity**

The Empire Some of the most significant organisms are fungi. They keep the cycle of nutrients across ecosystems going by decomposing decayed organic matter. Additionally, the symbiotic mycorrhizae that live in the roots of most vascular plants and provide vital nutrients are important to their growth. Numerous medicines (including penicillin and other antibiotics), meals like mushrooms, truffles, and morels, as well as the bubbles in bread, are all produced by other fungus.

In addition to causing human diseases like ringworm and athlete's foot, fungi also cause a variety of diseases in plants and animals. Fungal infections are especially challenging to treat because fungi are more chemically and genetically similar to animals than other creatures. But some fungi, particularly the yeasts, serve as crucial "model organisms" for research into genetic and molecular biology issues.

Antifungal medications are prescribed to patients with fungal infections. Antifungal medications, such antibiotics, prevent the fungus from growing naturally.

The antifungal medications currently employed to treat fungi infections are divided into:

- 1) Polyenes- acts by binding to ergosterol i.e. membrane sterols. e.g. Amphotericin B, Nystatin.
- 2) Azole derivatives-acts by inhibiting ergosterol biosynthesis. e.g. Miconazole, Ketoconazole, Fluconazole and Itraconazole.
- 3) Nucleotide-act by inhibiting DNA and RNA synthesis. e.g. 5-Fluorocytosine
- 4) Grisons-acts by inhibiting microtubular function. e.g. Griseofulvin.
- 5) Allylamines-e.g. Naftifine, Terbinafine.
- 6) Thiocarbamates-e.g. Tolnaftate.
- 7) Morpholines-e.g. Amorolfine.

Some herbs used as antifungal agents:

- Garlic is best antiseptics in general and antifungal.
- Licorice (*Glycyrrhiza glabra*).
- Teatree (*Melaleuca*, various species)
- Black walnut (*Juglans nigra*).
- Camomile (*Matricaria recutita*).

II. MATERIAL AND METODS

Anthelmintic activity

Animal

Indian adult earthworms (*pheretima posthuma*) were used to study anthelmintic activity. The earthworms were coected from moist soil and washed to remove all fecal materials. The earthworms 3-5 cm. in length and 0.1 – 0.2 cm in width were used for all experimental protocol. The earthworms resembles both automatically and physiologically to the intestinal roundworms parasites of human beings, hence can be used to study anthemintic activity.

Drugs and Chemicals

Albendazole suspension, Saline water, vehical (0.5% Acacia in Saline water)

Preparation of suspension

The suspension of ethanolic extract of leaves of *Cocos nucifera* (L.) of different cocentrations (25,50,100 mg/ml) were prepared by using 0.5% w/v Acacia as a suspending agent and final volume made up to 15 ml for respective concentration. Albendazole was used as a standard. Group of approximately equal size worms consisting of six earthworms individually in each group were released into in each 15 ml of desired concentration of drug and extracts in the petridish.

Anthelmintic Activity

The crude extract obtained was tested for Anthelmintic activity. *Pheretima posthuma* (earthworms collected from farm near river area of Haripur, Sangli) of nearly equal size ($5\text{cm} \pm 1$) were used for the in vitro Anthelmintic activity. The worms were acclimatized to the laboratory condition before experimentation. The worms were divided into respective groups containing six earthworms in each group. All the crude extract were dissolved in minimum quantity of 0.5% acacia and final volume adjusted to 15 ml with normal saline for making concentrations 25, 50, 100 mg/ml. All extract solutions and standard drug solution were freshly prepared before commencement of experiments. All the earthworms were washed in normal saline solution before they were released into 15 ml of respective formulation as follows, (0.5%acacia in normal saline), Albendazole and plant extract (25,50 and 100 mg/ml). Six earthworms of about the same size per pertidish were used. They were observed for their spontaneous motality and evoked responses. Observations were made for the time taken to paralysis and death of of individual worm. Paralysis was said to occure when the worms do not revive in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour.

Statistical Analysis

Result were expressed as mean \pm S.E.M. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test, with the level of significance at $P < 0.001$, $P < 0.01$ and $P < 0.05$

Antimicrobial activity

Antibacterial activity

Chemicals

Ethanolic extract of *Cocos nucifera* (L) leaves, Amoxicillin, Mueller Hinton Agar powder, DMSO, Distilled water, Nutrient broth

Bacteria

Staph. aureus, E.coli

Preparation of sample

The powder of ethanolic extract of leaves of *Cocos nucifera* (L) and amoxicillin were separately dissolved in dimethylsulfoxide (DMSO) to get concentrations of 500 ug/ml and 1000 $\mu\text{g/ml}$.

Sterilization of equipment and chemicals

Mueller Hinton agar, Nutrient broth, petri plates, cork borer, cotton swabs, variable micropipette were sterilized in autoclave at 15 lbs pressure (121°C) for 15mins.

Method

Agar well plate diffusion method

Procedure

The media were sterilized in autoclave at 121°C and 15 lbs pressure for 15 mins. Then cooled it up to 45°C and poured in petri plates with 6mm of thickness. Known concentration (0.1 ml) of inoculum were spread on the surface of solidified agar with spread plate technique. Wells were formed by using sterile cork borer. Different concentration of extract and antibiotics (500 µg/ml and 1000µg/ml) were pour into wells formed on the agar plate. Incubate it for 24hrs at 37°C and zone of inhibition were checked.

Antifungal activity

Procedure:

Antifungal activity against *Candida albicans*

(Agar well plate diffusion Method)

Stock solution for antifungal activity:

For antifungal study sample concentration of 5mg and 10 mg stored in a refrigerator till further used. Antifungal activities of the sample were evaluated by means of agar well diffusion assay. The assay was carried out according to the method of (Hufford *et al.*, 1975). Sabouraud dextrose agar (Hi media) was used for the growth of fungus. Media with acidic pH (pH 5.5 to 5.6) containing relatively high concentration of glucose (40%) is prepared by mixing (SDA) Sabouraud dextrose and distilled water and autoclaved at 121°C for 15 minutes. Twenty five ml of molten (45°C) SDA medium was aseptically transferred into each 100mm×15mm sterile Petri dish.

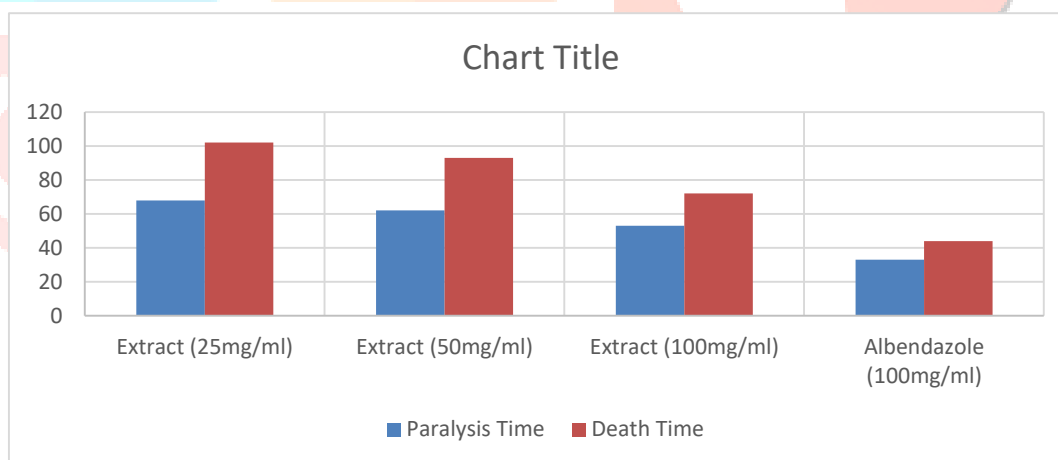
For counting of spore (fungi) were suspended in normal saline to make volume up to 1ml and then counted with help of hemacytometer (neubar chamber). Once the agar was hardened, 6mm wells were bored using a sterile cork borer. Then 0.1ml (100µl) from each stock solution of the sample having final concentration of 5 mg and 10mg was placed in each the well and the plates were incubated for 72 hour at 29°C.

The antifungal activity was measured as the diameter (mm) of clear zone of growth inhibition.

III. RESULT AND DISCUSSION

Result for Anthelmintic activity

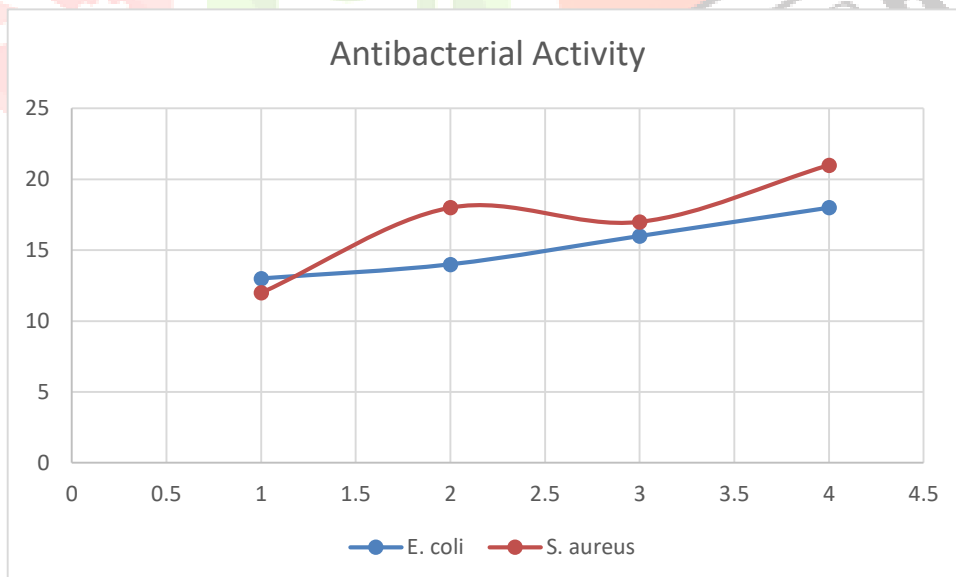
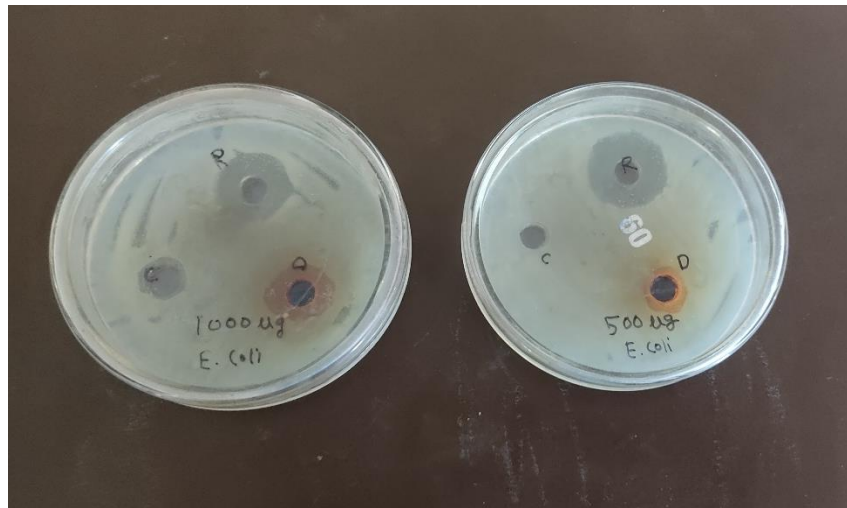
Concentration (mg/ml)	Paralysis Time	Death Time
Extract (25mg/ml)	68 min	102 min
Extract (50mg/ml)	62 min	93 min
Extract (100mg/ml)	53 min	72 min
Albendazole (100mg/ml)	33 min	44 min
Control	-	-



Result for Antimicrobial activity

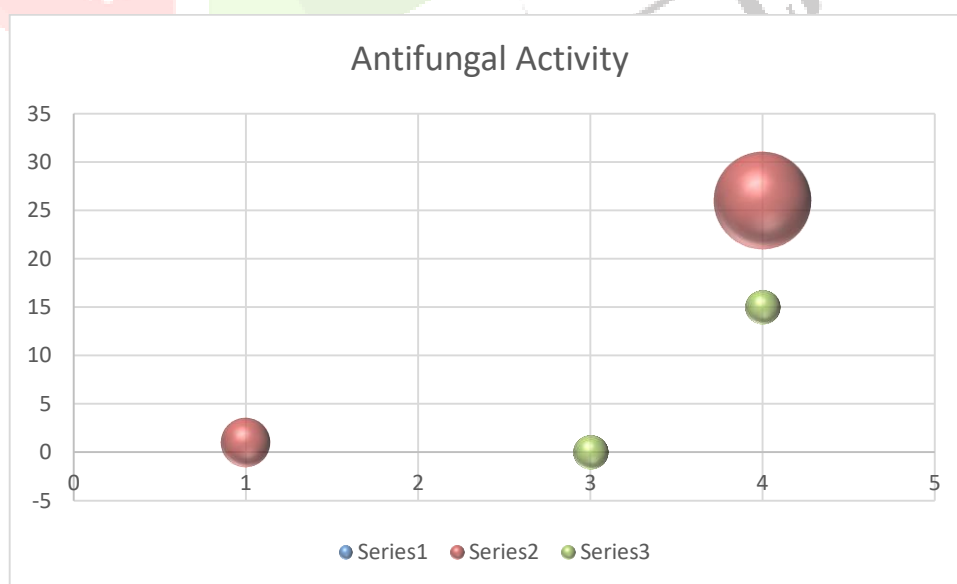
a) Result for anti-bacterial activity of sample against bacteria

Sr. No.	Bacteria	Zone of Inhibition (mm)			
		500 µg/ml		1000 µg/ml	
		Extract	Reference	Extract	Reference
1	E. coli	13 mm	14 mm	16 mm	18 mm
2	S. aureus	12 mm	18 mm	17 mm	21 mm



b) Result for anti-fungal activity of Samples against *Candida albicans*

Sr. no.	Samples	Conc. (mg/ml)	Zone In Diameter(mm) against <i>Candida albicans</i>
1	Control	-	26
2	Sample	5mg	08
		10 mg	15



IV. CONCLUSION

The ethanolic extract of *Cocos nucifera* (L.) leaves exhibits good anthelmintic activity against reference drug Albendazole, significant antibacterial activity against gram positive and gram negative bacteria and good antifungal activity against candida albicans. Therefore we can conclude that *Cocos nucifera* (L) leaves have significant antihelminthic and antimicrobial potential.

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