

**IN- VITRO ANTIBACTERIAL EVALUATION, ANTIOXIDANT ACTIVITY OF
LEMON PEEL AND ITS ACTIVATED CHARCOAL AND THEIR TOOTH
ANTISTAINING ACTIVITY FOR ORAL HYGIENE**

Submitted in partial fulfillment of the requirements for the award of

Bachelor of Pharmacy

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DECLARATION

We do hereby declare that the work presented in the thesis entitled "**IN-VITRO ANITBACTERIAL EVALUATION,ANTIOXIDANT ACTIVITY OF LEMON PEEL AND ITS ACTIVATED CHARCOAL AND THEIR TOOTH ANTISTAINING ACTIVITY FOR ORAL HYGIENE**", submitted by me to the **SATHYABAMA INSTITUTE OF SCIENCE & TECHNOLOGY**, Chennai-600119 for the award of the degree of **Bachelor of Pharmacy (2019-2023)** by Research is an original record of research work carried out by me under the supervision of **Dr. V. AMUDHAVALLI M.Pharm., Ph.D., Associate Professor, Department of Pharmacy**". The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma, membership, fellowship, associateship etc... In keeping with the general practice in reporting scientific observation, due acknowledgement has been made whenever the work described is based on the findings of other investigators.

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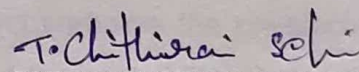
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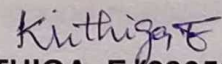
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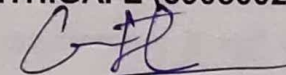
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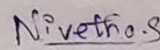
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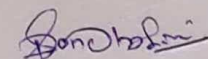
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ABSTRACT

Natural products have been recently investigated more thoroughly as promising agents for the prevention of oral diseases; especially plague related diseases such as dental carries. Oral diseases, a major health issue in the world, are economically affecting people of developed countries as 10% of the health expenditure is related to dental care. Antibacterial activity was done by using the compound material 1,2, and 3 (LC and AL+C). The compound material was prepared by using DMSO and the test pathogen was prepared by using bacterial inoculum. Using well diffusion method, the antibacterial activity was performed. The sample LC showed 15mm of zone of inhibition against gram positive *staphylococcus aureus*. Antioxidant activity was done by compound material 1,2, and 3 (LC, LP and L+Ci). The compound material was prepared by using methanol, acetone and water as solvent. Using hydrogen peroxide method, various concentrations were taken (LC,LP and L+Ci), ascorbic acid as control,. The LP+Ci compound material given a good antioxidant activity. Antistaining activity using methylene blue as control and compound material 1,2 and 3(LC, LP and L+Ci) with water. On 0.5 concentrations (10 to 100 µg/ml) of methylene blue, the increasing concentrations of compound material 1,2 and 3 given a good ant staining activity. Tooth antistaining activity was done by using compounding material 1,2 and 3 (LC, LP and L+Ci) was prepared with water and using soaking method and mechanical brushing method, the tooth' stains was moderately removed. The physiochemical method was done by performing solubility test using methanol, acetone and water for compound material 1,2 and 3 and it was not soluble. Then FTIR was carried out to analyse and compare the physiochemical properties of chosen herbal compound materials 1,2 and 3 (LC, LP and LC+Ci). Thus the present study showed that LP and L+Ci herbal compound material is a promising drug for the Oral hygiene .And the studies paving several way to bring a product for oral hygiene with a special quality standard requirement it will be adopted in future studies .

LIST OF ABBREVIATION

1.	DPPH	1,1-diphenyl-2-picrylhydrazyl (DPPH)-2,2-diphenyl-1-picrylhydrazyl
2.	FT-IR	FT-IR stands for “Fourier transform infrared” and it is the most common form of infrared spectroscopy
3	UV	Ultraviolet spectroscopy
4	L+Ci	Lemon peel with cinnamon
5	AL + Ci	Activated charcoal with cinnamon
6	MHA	Muller Hinton agar
7	MHB	Muller Hinton broth
8	DMSO	Dimethyl sulfoxide
9	LC	Dried lemon peel with charcoal
10	LP	Dried lemon peel powder
11	L+Ci	Dried lemon peel powder with cinnamon powder
12	MB	Methylene blue concentration
13	LC+Ci	Dried Lemon Peel Charcoal + Cinnamon Powder.

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CHAPTER-1

1. INTRODUCTION

1.1. ORAL HYGIENE

Oral health is an integral part of overall health, and each influences the other. Improper diet, smoking, alcohol intake, and poor oral hygiene practices are the most significant factors influencing the occurrence of various oral diseases.

Diet affects the development of dental caries, dental erosion, periodontitis, oral cancer, and many other diseases of the soft tissues of the oral cavity. Smoking has been linked to oral cancer, gingival and periodontal disease, periimplantitis, tooth discoloration, halitosis, taste bud changes, and difficulty healing wounds after surgery. Poor oral hygiene can lead to the development of dental caries and periodontitis, and is also associated with heart disease, cancer, and diabetes.

Many oral diseases are preventable through education about risk factors. Oral hygiene is a critical factor in maintaining good oral health, and subsequently is related to overall health and quality of life. The most effective method for preventing dental caries or periodontitis is the removal of dental plaque by regular and proper mechanical cleaning of the teeth, a key step in maintaining oral health. Knowledge of oral health is a fundamental prerequisite for healthy behavior, which allows an individual to take measures to protect their own health.

Is Herbal medicine the right choice for oral health?

Plant species have long been regarded as possessing the principal ingredients used in widely disseminated ethno medical practice. Herbal medicines considered to be safe and effective alternatives compared to synthetic chemicals. Plant extracts are gaining high interest due to their low toxicity and less side effects all over the world. Herbal drugs were statistically efficacious in controlling plaque and gingivitis with potent antimicrobial activity.

1.1.1. Herbal extract on oral health

Achillea millefolium L. (Asteraceae)

Camellia sinensis (L) Kuntze (Theaceae)

Aloe vera (L) Burn.f. (Xanthorrhoeaceae)

Catha edulis forsk (Celastraceae)

Citrus aurantifolia swing (Rutaceae)

Commiphora myrrha Mr.E.M. Holmes (Burseraceae)

Curcuma longa L (Zingiberaceae)

Gaultheria procumbens L(Ericaceae)

Hypericum perforatum L(Hypericaceae)

Lippia sidoides cham (Verbenaceae)

Matricaria chamomilla L (Asteraceae)

Menthe piperita L (Lamiaceae)

1.1.2. Use of herbal extract

Herbal extracts have been used in oral health for reducing inflammation. They act as antimicrobial plaque agents for preventing release of histamine. They also act as antiseptics, antioxidants, antimicrobials antivirals and analgesics.

Oral health is an essential aspect of general health can be defined as “a standard of health of the oral and related tissues which enables an individual to eat, speak, and socialize without active disease, discomfort or embarrassment and which contributes to general well- being”.

The link between oral diseases and the activities of microbial species that form part of the micro biota of the oral cavity is well established.

Over 750 species of bacteria inhabit the oral cavity (~50% of which are yet to be identified) and a number of these are implicated in oral diseases.

Over 750 species of bacteria inhabit the oral cavity (~50% of which are yet to be identified) and a number of these are implicated in oral diseases.

The development of dental caries involves acidogenic and aciduric Gram-positive bacteria, primarily the mutans streptococci (*Streptococcus mutans* and *S.sobrinus*), lactobacilli and actinomycetes, which metabolize sucrose to organic acids (mainly lactic acid) that dissolve the calcium phosphate in teeth, causing decalcification and eventual decay.

Dental caries is thus a supragingival condition. In contrast, periodontal diseases are subgingival conditions that have been linked to anaerobic Gram-negative bacteria such as *Porphyromonas gingivalis*, *Actinobacillus sp.*, *Prevotella sp.* and *Fusobacterium sp.* In periodontal diseases, the areas at or below the gingival crevice become infected causing a cellular inflammatory response of the gingiva and surrounding connective tissue.

The global need for alternative prevention and treatment options and products for oral diseases that are safe, effective and should be a traditional medicine.

Table 1.1 List of whitening agents and its mechanism of action

Whitening agent	Mode of action
Abrasives (e.g., hydrated silica, perlite, alumina) most important toothpaste ingredient for stain removal.	Mechanical removal of extrinsic stains.
Antiredeposition agents (e.g., polyphosphates, sodium citrate)	Prevention of the deposition of chromophores and inhibition of calculus formation where external stains could be incorporated
Calcium phosphates (eg: hydroxyapatite)	Adhesion of white calcium phosphate particles on the tooth surface and prevention of bacterial attachments/plaque -

	formation on the teeth
Colorants (eg:blue covarine)	Shifting color absorption and reflection spectra from yellow to blue
Enzymes/proteases (eg:papain, bromelain)	Support stain removal due to degradation of proteins (hydrolysis of peptide bonds)
Peroxides (eg:hydrogen peroxide, calcium peroxide)	Oxidation of organic chromophores
Polyaspartate (eg: sodium polyaspartate)	Inhibition of plaque-formation
Surfactants (eg: sodium laurylsulfate)	Removal of hydrophobic compounds from the tooth surface

Hence the research needs for the alternative, & deep learning the sources of traditional medicine are considered and it acts as a good alternative to synthetic chemicals.

1.2 LEMON PEEL

Synonym

Fructus Limonis.

Biological source

Lemon peel is obtained from the fresh ripe fruit of *Citrus limon* (L.) Burm. f. (*C. medica* var. *limon* Linn).

Family

Rutaceae

Chemical Constituents

Lemon peel contains volatile oil (2.5%), vitamin C, hesperidin and other flavone glycosides, mucilage, pectin and calcium oxalate. The important constituents of the volatile oil are limonene (90%), citronellal, geranyl acetate, α -pinene, camphene, linalool, terpineol, methyl heptenone, octyl and nonyl aldehydes, γ -terpinene, β -pinene, neral, and geranial.

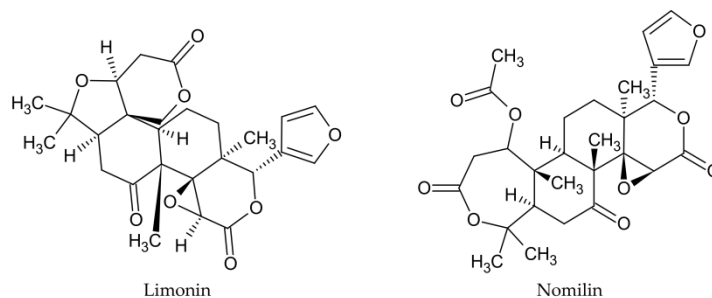


Figure 1.1. Structure of Limonin and Nomilin

The peels also contain flavonoids eriocitrin, epigenin, luteolin, chrysoeriol, quercetin, isorhamnetin, limocitrin, limocitrol, isolimocitrol, hesperidin; coumarins scopoletin and umbelliferone, sinapic acid and β - coumaric acid.

Uses

Lemon peel is used as a flavouring agent, perfumery, stomachic, and carminative. The oil, externally, is a strong rubefacient and if taken internally in small doses has stimulating and carminative properties.

LEMON PEEL

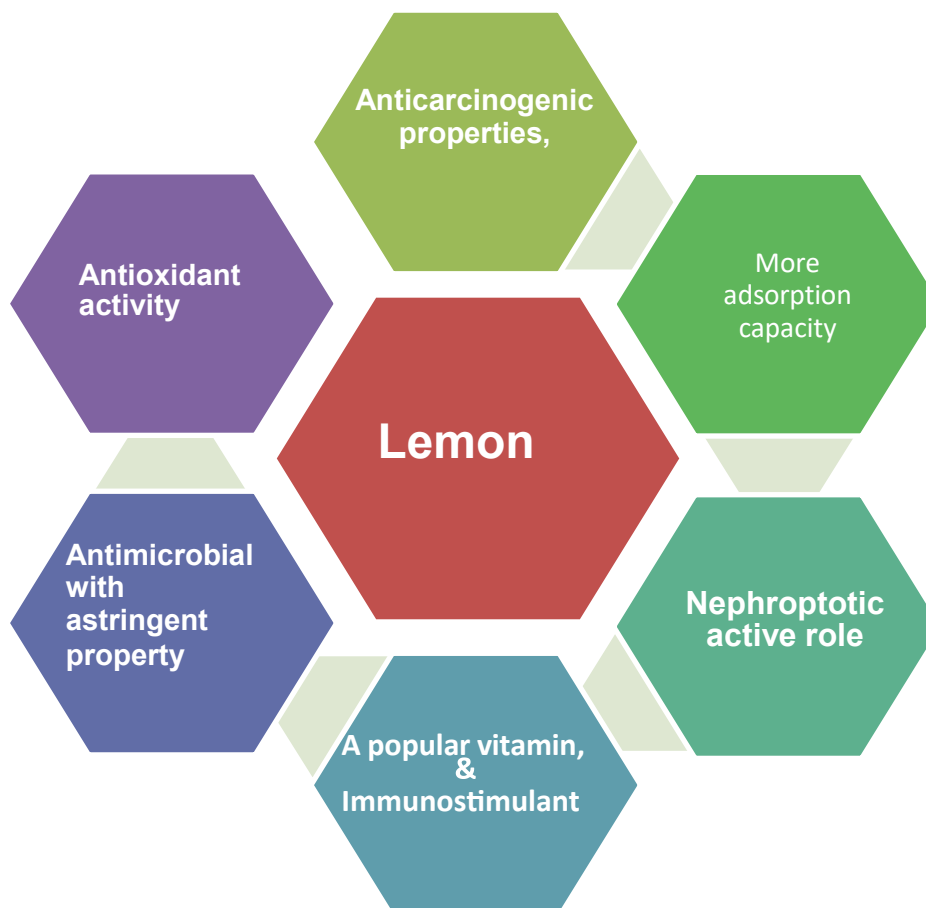


Figure 1.2. *Schematic representation of properties of lemon*

The peel of citrus fruits is a rich source Of flavanones an many polymethoxylated flavones, which are very rare in other plants. These compounds, not only play an important physiological and ecological role, but are also of commercial interest because of their multitude of applications in the food and pharmaceutical industries.

The citrus peel oils show strong antimicrobial activity which will be influenced in present study for their effective antimicrobial and anti-staining agent in oral health disorders.

Vitamin C deficiency has been linked to a variety of oral health problems like gingivitis and bleeding gums, so the robust vitamin C content in lemon peel can help avert such a deficiency. However, there are also antimicrobial properties of lemon peel that can help prevent gum infections.



Figure 1.3 *Picture representation of dried lemon peel*

Citrus limon is a potent medicinal plant belonging to family *Rutaceae* originated from the tropical and subtropical Southeast Asia, is the 3rd most beneficial citrus specie next to orange and mandarin. The genus citrus consists of about 1300 species and 140 genera. Globally, the citrus production is about 105 million metric tons per year.

The fruit contains high amounts of nutrients and provides wider range of health benefits when consumed as juice.

A broad spectrum of biological activity like (antibacterial, antidiabetic, antioxidant anticancer, antifungal, antiviral activities.)

We have isolated 4 antibacterial substances that were active against the oral bacteria that cause dental caries and periodontitis, such as *Streptococcus mutans*, *Prevotella intermedia*, and *Porphyromonas gingivalis*, from lemon peel, a waste product in the citrus industry.

Lemon peels utilizable foodstuffs were analysed for crude protein, phosphorus, calcium, copper, manganese, iron, zinc, sodium, potassium and some chemical parameters in feedstuffs. The levels of protein (9.42 %), fat (4.98%), ash (6.26%), fiber (15.18%), sodium (755.5 mg/100g), potassium (8600 mg/100g), calcium (8452.5 mg/100g), copper (4.94 mg/100g), iron (147.65 mg/100g), magnesium (1429.50 mg/100g), zinc (13.94 mg/100g) and phosphorus (6656 mg/100g) detected in the lemon peels.

1.3. ACTIVATED CHARCOAL OF DRIED LEMON PEEL

Activated carbon contains carbonaceous material derived from charcoal. Activated carbon is produced by pyrolysis of organic materials of plant origin.

These materials include coal, coconut shells and wood, sugarcanebagasse, soybean hulls and nutshell.

Activated carbon is used to treat poisonings and overdoses following oral ingestion. Tablets or capsules of activated carbon are used in many countries as an over-the-counter drug to treat diarrhea, indigestion, and flatulence.

Activated carbon is usually derived from waste products such as coconut husks; waste from paper mills has been studied as a source. These bulk sources are converted into charcoal before being 'activated'. When derived from coal. It is referred to as activated coal. Activated coke is derived from coke.

1.3.1. Activated charcoal uses

Activated charcoal is primarily used to decolorize wine or remove off-odors. Activated charcoal that can be used in water purification processes. Activated charcoal treatment at water treatment plants is typically installed to provide removal of natural organic compounds, taste and odor compounds, and synthetic organic chemicals. Activated charcoal filters are used for de-chlorination of water, adsorption and H₂S removal.

1.4 CINNAMON

Cinnamon bar

Synonyms

Cortex cinnamon. Ceylon cinnamon. Saigon cinnamon. Chinese cassia.
Cinnamomum aromaticum. Cinnamomum laurus.

Biological Source

Cinnamon is the dried inner bark of the coppiced shoots of *Cinnamomum zeylanicum* Nees.

Family

Lauraceae

Chemical Constituents

innamon contains about 10% of volatile oil, tannin, mucilage, calcium oxalate and sugar. Volatile oil contains 50 to 65% cinnamic aldehyde, along with 5 to 10% eugenol, terpene hydrocarbons and small quantities of ketones and alcohols.

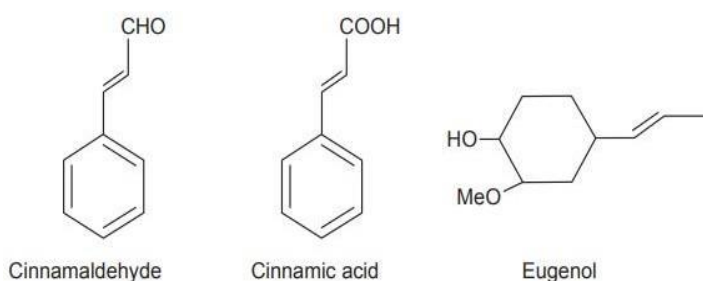


Figure 1.4 Structure of Cinnamon

Uses

It is used as an alternative aromatic, carminative, flavouring agent, analgesic, antiseptic, antirheumatic, antispasmodic, demulcent, digestive, expectorant, stomachic, diaphoretic, antibacterial, antifungal, etc. It stops vomiting, relieves flatulence and is given with chalk and as astringents for diarrhea and haemorrhage of the womb. It is also used in the treatment of bronchitis, colds,

palpitations, nausea, congestion, and liver problems.



Figure1.5 Pictorial representation of dried cinnamon bark

Cinnamon consists of dried bark, freed from the outer cork and from the underlying parenchyma, from the shoots growing on the cut stumps of *Cinnamomum zeylanicum* Nees.

The cinnamon is generally cultivated by seed propagation method but sometimes plant cuttings are also preferred. It mainly needs sandy or siliceous soil which should be rich in humus. The other requirements for its better cultivation are altitude (800 to 1000 meter) and annual rainfall (200 to 250 cm). It is a shade loving plant. The seeds are propagated in nursery beds in the month of June and July.

The bark of various cinnamon species is one of the most important and popular spices used worldwide not only for cooking but also in traditional and modern medicines.

Overall, approximately 250 species have been identified among the cinnamon genus, with trees being scattered all over the world. Cinnamon is mainly used in the aroma and essence industries due to its fragrance, which can be incorporated into different varieties of foodstuffs, perfumes, and medicinal products. (Pharmacognosy book).

The most important constituents of cinnamon are cinnamaldehyde and *trans*-cinnamaldehyde (Cin), which are present in the essential oil, thus contributing to the fragrance and to the various biological activities observed with cinnamon.

A study on *Cinnamomum osmophloeum* (*C. osmophloeum*) indicated that the essential oil from cinnamon leaves contains a high level of Cin. Consequently, *C. osmophloeum* is also used as an alternative spice for *C. cassia*. One of the major constituents of essential oil extracted from *C. zeylanicum* named (E)-cinnamaldehyde has an antityrosinase activity, while cinnamaldehyde is the principal compound responsible for this activity. Cinnamon bark contains procyanidins and catechins. These procyanidins extracted from cinnamon and berries also possess antioxidant activities.

The antimicrobial effects of cinnamon essential oil (EO), cinnamon extracts, and pure compounds against different oral pathogens and the oral biofilm and the possible effects on soft mouth tissue. Basic information is provided about cinnamon, as is a review of its antimicrobial properties against the most common microorganisms causing dental caries, endodontic and periodontal lesions, and candidiasis.

1.5. ANTIBACTERIAL ACTIVITY

Antibacterials are used to treat bacterial infections. Antibiotics are classified generally as beta-lactams, macrolides, quinolones, tetracyclines or aminoglycosides.

Their classification within these categories depends on their antimicrobial spectra, pharmacodynamics, and chemical composition. Prolonged use of certain

antibacterials can decrease the number of enteric bacteria, which may have a negative impact on health.

Consumption of probiotics and reasonable eating may help to replace destroyed gut flora. The discovery, development and use of antibacterials during the 20th century have reduced mortality from bacterial infections.

The antibiotic era began with the therapeutic application of sulfonamide drugs in 1936, followed by a “golden” period of discovery from about 1945 to 1970, when a number of structurally diverse and highly effective agents were discovered and developed.

A variety of laboratory methods can be used to evaluate or screen the in vitro antimicrobial activity of an extract or a pure compound. The most known and basic methods are the disk-diffusion and broth or agar dilution methods.

1.5.1. METHODS OF ANTIBACTERIAL ACTIVITY

Diffusion methods

Agar disk-diffusion method

Agar disk-diffusion testing developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts testing. Although not all fastidious bacteria can be tested accurately by this method, the standardization has been made to test certain fastidious bacterial pathogens like streptococci, Haemophilus influenzae, Neisseria gonorrhoeae and Neisseria meningitidis, using specific culture media, various incubation conditions and interpretive criteria for inhibition zones.

Antimicrobial gradient method (Etest)

The antimicrobial gradient method combines the principle of dilution methods with that of diffusion methods in order to determine the MIC value. It is based on the possibility of creating a concentration gradient of the antimicrobial agent tested in the agar medium.

Agar well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts.

Agar plugs diffusion method

Agar plugs diffusion method is often used to highlight the antagonism between microorganisms.

Cross streak method

Cross streak method is used to rapidly screen microorganisms for antagonism.

Dilution methods:

Dilution methods are the most appropriate ones for the determination of MIC values, since they offer the possibility to estimate the concentration of the tested antimicrobial agent in the agar (agar dilution) or broth medium (macrodilution or microdilution). Either broth or agar dilution method may be used to quantitatively measure the in vitro antimicrobial activity against bacteria and fungi.

(a) Broth diffusion method

Broth micro- or macro-dilution is one of the most basic antimicrobial susceptibility testing methods.

(b) Agar diffusion method

This technique is suitable for both antibacterial and antifungal susceptibility testing.

1.6. ANTIOXIDANT ACTIVITY

Antioxidant means "against oxidation". Any substance at low concentrations compared to that of an oxidizable substrate that significantly delays or prevents oxidation of that substrate is called as antioxidant. Antioxidants play vital role in preserving the quality of food and maintaining health of human being.

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Herbal plants considered as good antioxidant since ancient times.

1.6.1. CLASSIFICATION OF ANTIOXIDANT

Broadly, there are five major types of antioxidants as described below:

Primary antioxidants or chain breaking antioxidants are those compounds, mainly phenolic substances that terminate the free radical chains in lipid oxidation and function as hydrogen and electron donors. In addition, primary antioxidants chelate transition metals acting as catalyst in lipid oxidation. Oxygen scavengers are those substances which react with oxygen and can thus remove it in a closed system, e.g., ascorbic acid (vitamin C).

Secondary antioxidants are those compounds which function by decomposing the lipid hydroperoxides into stable end products.

Enzymatic antioxidants are those enzymes which function either by removing dissolved or head space oxygen, e.g., glucose oxidase, or by removing highly oxidative species, e.g., super oxide dismutase.

Chelating agents are synergistic substances which greatly enhance the action of phenolic antioxidants. Most of these synergists exhibit little or no antioxidant activity, for example citric acid, amino acid, and phospholipids such as cephalin.

1.6.2. METHODS OF ANTIOXIDANT ACTIVITY:

1.6.2.1. Hydrogen peroxide method:

Varying quantities of the stock solution was added to 3.8ml of 0.1M phosphate buffer solution (PH 7.4) and then mixed with 0.2ml of hydrogen peroxide solution. The absorbance of the reaction mixture was measured at 230nm after 10min. The mixture without sample was used as blank. Ascorbic acid was used as standard. The percentage inhibition of hydrogen peroxide will be calculated using the formula.

$\% \text{inhibition} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100.$

1.6.2.2. DPPH method

One mL of peel extract was taken in various concentrations (50-300 µg/mL) and was mixed with 1 mL of 0.1 mM of DPPH solution in methanol. The reaction mixture was kept at room temperature for 30 minutes. Absorbance was read at 517 nm in spectrophotometer.

The percentage of the radical scavenging activity was calculated as follows:

$\text{Absorbance in control} - \text{Absorbance in sample} \times 100 / \text{Absorbance in control}$

1.7. ANTISTAINING ACTIVITY

Tooth discoloration is a major oral health concern and has been seen to affect the quality of life of an individual. While the majority of intrinsic tooth discoloration is due to ingested chemical or drug taken during teeth forming years; extrinsic stains are mostly related to the individual's personal harmful practices which include smoking, tobacco chewing, beetle leaves (paan), areca nut chewing and dietary habits.

There is a wide and high level of interest in products which could have some stain-removing effect. Over the years, scientists have compared chemical and mechanical approaches for stain removal. Studies of chemical agents have focused on chelants, surfactants, and enzymes which could prevent or remove extrinsic dental stains. Various ingredients that could assist in the stain-removal process have been tested, but none of them have been indicated as a potential addition to food due to their toxicity. Consequently, controlled chemical, non- abrasive agents like chewing gum or mouthwash are being favored as a delivery vehicle for these stain removing ingredients.

On the other hand, mechanical approaches have focused on tooth brushing. It is established that tooth-brushing is the most commonly used methods of oral hygiene maintenance. It has been noted that tooth-brushing with dentifrices helps decrease plaque and calculus deposits on teeth as well as support in eradicating stains and discolorations. It must be realized that stains are deposition of a variety of pigments originating from the diet or tobacco use on the tooth surface. This deposition can be reduced by effective oral hygiene practices, which focuses on toothbrushes and dentifrices. The dental professionals need to know the actual efficacy and the correct indications of the available products in order to recommend one appropriate to the individuals and the communities. Studies like the present may answer these questions in order to guide consumers and professionals in using oral home-care products. Tooth staining can have an adverse psychosocial effect on a patient and can create an esthetic challenge for the clinician.

According to the Glossary of Prosthodontic Terms, 'stain' has been defined as the discoloration of a tooth surface or surface as a result of ingested materials, bacterial action, tobacco, and/or other sub-stances. This may be intrinsic, extrinsic, acquired, or developmental. Vogel introduced two categories for tooth discoloration as extrinsic or intrinsic and later Watts introduced a third category called internalized discoloration. This classification depends on specific etiologica factors. Several factors affect the color of the tooth as seen by the human eye, some of which include: tooth's physi-ologic components (enamel, dentin and pulp), gradation of color based on specific location on the tooth (gingival shades are darker than incisal translucency), individual teeth (central and lateral incisors are lower in value than canines), age of the patient (teeth are lighter at a younger age than at an older age due to physiologic wear of enamel, secondary dentin deposition and staining), quality of the incident and reflected light (determining how the tooth is eventually viewed). Determination of the etiologic factor causing staining plays an important role in planning a specific treatment modality and its related outcome.

According to our study we have done methylene blue dye adsorption method for tooth antistaining activity. The adsorption method of methylene blue shows good discoloration for our samples by using different concentration levels at regular interval of time in seconds for drug sample.

The antistaining activity for tooth in our study is done by two methods:

- Soaking method.
- Mechanical brushing method.

1.8 FTIR

FTIR stands for "Fourier transform infrared" and it is the most common form of infrared spectroscopy. All infrared spectroscopies act on the principle that when infrared (IR) radiation passes through a sample, some of the radiation is absorbed. The radiation that passes through the sample is recorded. Because different molecules with their different structures produce different spectra, the spectra can be used to identify and distinguish among molecules. In this way, the spectra are virtually unique.

FTIR is the preferred method of infrared spectroscopy for several reasons. First, it does not destroy the sample. Second, it is significantly faster than older techniques. Third, it is much more sensitive and precise.

1.8.1 Use

FTIR was used for the detection of Physiochemical properties from Jeppiaar research park from Sathyabama Institute of Science and Technology.

1.9 UV Spectroscopy

spectroscopy is related to the interaction of light with matter. As light is absorbed by matter, the result is an increase in the energy content of the atoms or molecules. When ultraviolet radiations are absorbed, this results in the excitation of the electrons from the ground state towards a higher energy state. Molecules containing π -electrons or nonbonding electrons (n-electrons) can absorb energy in the form of ultraviolet light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons, the longer the wavelength of light they can absorb. There are four possible types of transitions (π - π , n- π , σ - σ , and n- σ), and they can be ordered as follows: σ - $\sigma^* > n$ - $\sigma^* > \pi$ - $\pi^* > n$ - π^* . The absorption of ultraviolet light by a chemical compound will produce a distinct spectrum that aids in the identification of the compound.

1.9.1 Use

The shimadzu spectrophotometer type -1900 was used for analyzing the antioxidant activity.

CHAPTER-2

2.REVIEW OF LITERATURE

2.1 ANTIBACTERIAL ACTIVITY

1) **Yoshiaki Miyake.**, et al. **(2011)** developed an Isolation and extraction of antimicrobial substances against oral bacteria from lemon peel. The studies - involved the isolation 4 antibacterial substances that were active against the oral bacteria that cause dental caries and periodontitis, such as *Streptococcus mutans*, *Prevotella intermedia*, and *Porphyromonas gingivalis*, from lemon peel, a waste product in the citrus industry. The isolated substances were identified as 8- geranyloxypsolaren, 5-geranyloxypsolaren, 5-geranyloxy-7-methoxycoumarin, and phloroglucinol 1- β -D-glucopyranoside (phlorin) upon structural analyses. Among these, 8-Geranyloxypsolaren, 5 geranyloxypsolaren, and 5-geranyloxy-7- methoxycoumarin exhibited high antibacterial activity. These 3 compounds were effectively extracted using ethanol and n-hexane, whereas phlorin was extracted with water. Further, the above 3 compounds were present in lemon essential oil and abundantly present in the residue produced upon the cooling treatment of concentrated lemon essential oil.

2) **Da Silva.**,et al. **(2012)** determines an In vitro antimicrobial activity of mouth washes and herbal products against dental biofilm-forming bacteria

The study evaluated the in vitro, the antimicrobial effect of *Cymbopogon citrates* (lemon grass), *Plectranthusamboinicus* (Mexican mint) and *Conyzabonariensis* (hairy fleabane) tinctures as well as pure and diluted commercial mouth washes (Malvatricin®, Periogard® and Listerine®) on wild isolates of *Streptococcusmutans* and reference strains of *S. mutans*, *Streptococcus salivarius*, *Streptococcus oralis* and *Lactobacillus casei* by determination of minimum inhibitory dilution (MID). 0.12% chlorhexidine and 70% corn alcohol were used as positive and negative controls, respectively. Saliva samples were collected from 3 volunteers and seeded in MSB broth to obtain *Streptococcus* isolates after 72-hour incubation. Using the agar diffusion method, susceptibility tests were performed with overnight incubation in microaerophilia at 37°C. All tests

were performed in duplicate. The bacterial species were resistant to the tinctures and Listerine®, but were susceptible to 0.12% chlorhexidine, Malvatricin® and Periogard®, with MIDs ranging from 12.5% to 1.56%. They conclude *Plectrantusamboinicus*, *Conyzabonariensis* and *Cymbopogoncitratatus* tinctures and Listerine® did not show inhibitory action against the tested biofilm-forming bacteria.

4) Panel Najwa Nasser AL-Jabri., et al. (2014) performed a Comparative chemical composition and antimicrobial activity study of essential oils from two imported lemon fruits samples against pathogenic bacteria. The study described to isolate and identify two essential oils by hydro distillation method from two imported lemon fruits samples collected from local supermarket and evaluate their antimicrobial activity against pathogenic bacteria through disc diffusion method. The essential oil was obtained from Turkish and Indian lemon fruits samples by hydro distillation method using Clevenger type apparatus. Both isolated essential oils were identified by GC–MS and determine their in vitro antimicrobial activity against pathogenic bacteria through agar gel method. The predominant bioactive ingredients with high percentage in Turkish essential oil were dl-limonene (78.92%), α -pinene (5.08%), l- α -terpineol (4.61%), β -myrcene (1.75%), β -pinene (1.47%) and β -linalool (0.95%) and in Indian essential oil were dl-limonene (53.57%), l- α -terpineol (15.15%), β -pinene (7.44%), α -terpinolene (4.33%), terpinen-4-ol (3.55%), cymene (2.88%) and E-citral (2.38%) respectively. Both isolated essential oils by hydro distillation were used for the study of antimicrobial activity against four pathogenic bacterial strains such as *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*). Therefore, the obtained results show that both essential oils could be needed further extensive biological study and their mechanism of action.

5) Camilla Bringel Rego., et al (2016) developed a In vitro antimicrobial activity of essential oil of *Cymbopogon citratus* (lemon grass) on *Streptococcus mutans*. The study evaluated and investigated the effect of essential oil of lemon grass (LGO) on *Streptococcus mutans* biofilm developed in hydroxyapatite discs surface. Initially, the susceptibility of *S. mutans* to the LGO through the inhibition zone test in planktonic suspension and minimum inhibitory concentration (MIC)

was investigated. They evaluate the effect of the essential oil in biofilms, hydroxyapatite discs were used to simulate the tooth surface. The biofilms of *S. mutans* were developed on the discs for 5 days and immersed daily in the following groups: G1 - immersion for 5 min in LGO to 0808 mg/ml (test group) and G2 – Brain Heart Infusion (BHI) 1% Sucrose (negative control). Then biofilms were counted for colony forming unit (CFU) and transformed into log₁₀. The data were analyzed by ANOVA test with a P value <0.05. The susceptibility test was positive indicating inhibition of microorganisms and the MIC value was 0.04 mg/mL. As for biofilm results, it decreased the bacterial growth in G2 compared to G1 with a statistically significant difference (P <0.034). Considering the limitations of this study, it was concluded that the essential oil of lemon grass was effective in controlling bacterial growth in biofilms of *Streptococcus mutans*.

6) Sheila John.,et al. (2017) performed a Antioxidant and Antimicrobial Efficacy of Lemon (*Citrus limonum* L.) Peel. The study determined that waste materials such as peels and seeds obtained from post-harvest management are rich source of biologically active phytochemicals possessing various physiological functions. Usually these plant based products are often discarded as waste or utilized as feed or fertilizer. Nowadays the recent area of research interest includes exploiting the use of peels for the production of effectual, safe, inexpensive and novel nutraceuticals as they contain high amount of natural compounds with medicinal properties. The present study was carried out to determine the antioxidant and antimicrobial potential of lemon peel as it is a rich source of molasses, pectin, limonene and other secondary metabolites. The antioxidant activity of lemon peel was determined using DPPH assay, FRAP and phosphomolybdenum assay while the antibacterial activity was tested against four bacterial strains. The result indicated that acetone extract of lemon peel exhibited greater potential to scavenge free radicals and reducing power that increased with increase in concentration. Methanol extract of lemon peel showed greater antimicrobial activity thereby indicating the effectiveness of lemon peel as a potent antimicrobial agent. Hence, lemon peel functions as a potent antioxidant and antimicrobial agent and that in future its use as an ingredient in value added food supplements is promising. Keywords: lemon peel, phenolic compounds, nutraceutical property, antioxidant, antimicrobial.

7) **Raiane S Lemes., et al. (2018)** characterized a Chemical composition and antibacterial activity of essential oils from *Citrus aurantifolia* leaves and fruit peel against oral pathogenic bacteria. This study performed tooth decay was a major public health problem which affects a large number of people in several countries. Even though more than 700 bacterial species have been detected in the oral cavity, *Streptococcus* and *Lactobacillus* stand out as the genera that cause tooth decay and other periodontal diseases. In this study, essential oils from *Citrus aurantifolia* leaves (CL-EO) and fruit peel (CP-EO) were obtained by hydrodistillation by a Clevenger-type apparatus whereas their chemical composition was analyzed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Limonene (77.5 %), linalool (20.1 %), citronellal (14.5 %) and citronellol (14.2 %) were the main constituents found in the essential oils from *C. aurantifolia* leaves and fruit peel. Antibacterial activity of essential oils was evaluated in terms of its minimum inhibitory concentration (MIC) values by the broth microdilution method in 96-well microplates. Both CL-EO and CP-EO displayed some activity against all oral pathogens under investigation; MIC values ranged from 20 to 200 µg/mL. CL-EO and CP-EO not only had promising activity against *Streptococcus mutans* (MIC = 20 µg/mL) and *Lactobacillus casei* (31.25 µg/mL), but also displayed antibacterial activity against all studied cariogenic bacteria. Efficacy of essential oils against *S. mutans* and *L. casei* is noteworthy and should be further investigated.

2.2 ANTIOXIDANT ACTIVITY

- 1) **Hanan A Al-Ashaal ., et al. (2011)** describes a Antioxidant capacity of hesperidin from citrus peel using electron spin resonance and cytotoxic activity against human carcinoma cell lines. The study explained that Hesperidin is a flavonoid that has various pharmacological activities including anti-inflammatory, antimicrobial and antiviral activities. The work explains the isolation of hesperidin from the peel of *Citrus sinensis* L. (Rutaceae), and the evaluation of its antioxidant capacity and cytotoxicity against different human carcinoma cell lines. In the present work, hesperidin is identified and confirmed using

chromatographic and spectral analysis. To correlate between hesperidin concentration and antioxidant capacity of peel extracts, extraction was carried out using 1% HCl-MeOH, MeOH, alkaline solution, the concentration of hesperidin determined qualitatively and quantitatively using high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC) analysis, in vitro antioxidant capacity of hesperidin and the extracts against free radical diphenylpicrylhydrazyl (DPPH•) performed using an electron spin resonance spectrophotometer (ESR). Cytotoxic assay against larynx, cervix, breast and liver carcinoma cell lines was performed. The results conclude that Hesperidin was found to be moderately active as an antioxidant agent; its capacity reached 36%. In addition, the results revealed that hesperidin exhibited pronounced anticancer activity against the selected cell lines. IC₅₀ were 1.67, 3.33, 4.17, 4.58 µg/mL, respectively.

- 2) **José María García.**, et al. **(2015)** performed Dietary dehydrated lemon peel improves the immune but not the antioxidant status of gilthead seabream (*Sparus aurata* L. The study evaluated that Lemon (*Citrus limon*) is the third most important species of citrus in the world, while Spain is the major producer in Europe. Numerous beneficial effects of lemon are known, which explains their use in traditional medicine. The paper describes the effect of dietary dehydrated lemon peel (a sub-product of the lemon industry) on the growth, immune and antioxidant status of gilthead seabream (*Sparus aurata* L.) over a period of 30 days. Fish fed diets enriched with dehydrated lemon peel (1.5% and 3%) for 15 days showed improved growth and both humoral (seric immunoglobulin M) and cellular (peroxidase activity and phagocytic ability of head kidney leucocytes) immunity, as well as the expression of some immune-related genes (*nkef*, *il1β*, *igth* and *csfr1*). However, decreases growth promotion was observed after thirty days of trial. Neither the anti-oxidant enzymes activity nor the expression of several anti-oxidants and anti-stress genes in liver was improved by the diet. The possible inclusion of dehydrated lemon peel in fish diets for its immunostimulant effects is discussed.

3) Gizem Toprakçı, et al. (2022) developed a Highly clean recovery of natural antioxidants from lemon peels: Lactic acid-based automatic solvent extraction. The study determined that food industry generates large amounts of waste by-products rich in natural antioxidants. On the other hand, application of advanced processes for the recovery of these fine chemicals is another popular topic of recent years. The purpose of this study is to propose a green extraction method by application of deep eutectic solvent-based automated solvent extraction (AMSE) from lemon peels. The primary polyphenols (hesperidin, naringin, and p-coumaric acid) and the total polyphenols of the lemon peel extract were quantified and used as response for the optimisation of the AMSE conditions. The Box-Behnken design type of the response surface method (RSM) was chosen for optimisation study. Scavenging activity of the lemon peel extract against 2,2-diphenyl-1-picrylhydrazil (DPPH) free radical was also measured in vitro. The results conclude that the optimum conditions for the highest total phenolic (7.47 mg-gallic acid equivalent [GAE]/g-lemon peel [LP]), naringin (5.05 mg/g-LP), p-coumaric acid (3.27 mg/g-LP), and hesperidin (0.07 mg/g-LP) yields were obtained by 1.5 h of extraction time, 46% water (v/v), and 5 g of peel. The antioxidant activity changed between 37.31% and 94.10% in the peels. Extraction time was the most effective process factor for the total phenolic and p-coumaric acid yields, while water addition was statistically very important ($p < 0.0001$) for the naringin and hesperidin yields in the current AMSE system.

4) Angela Zappia, et al. (2023) performed a Evaluation of the Efficacy of Antioxidant Extract from Lemon By-Products on Preservation of Quality Attributes of Minimally Processed Radish (*Raphanus sativus* L.) This work enhanced the use of a food-grade antioxidant extract obtained from lemon processing byproducts (peel, pulp and seeds) to extend the shelf life of minimally processed radishes. The extract (LPE) was previously characterized in terms of total phenolic (6.75 ± 0.34 mg GAE g⁻¹ d.w.) and flavonoid content (2.04 ± 0.09 mg CE g⁻¹ d.w.) and antioxidant activity, and eriocitrin and hesperidin were identified as the most prevalent phenolic compounds by a UHPLC system. The effects of different dipping aqueous solutions (UCR, DRa, DRb) and alginate-based edible coating

formulations (CRc, CRd) with and without the antioxidant extract were studied on the quality parameters of minimally processed radishes, characterized regarding their microbiological and physicochemical characteristics for up to 14 days at 3 °C. The coating formulated with LPE delayed the radish respiration process, as well as resulting in less color variation ($\Delta E < 3$) and reduced mesophilic aerobic count values ($4.49 \pm 1.43 \log \text{CFU g}^{-1}$), proving the effectiveness of LPE as a value-added ingredient in developing post-harvest strategies to prolong the shelf life of minimally processed vegetables. Indeed, coated samples without the extract showed a clear development of rotting, which led to the end of their shelf life on their 7th day of storage.

5) Esther Gómez-Mejía ., et al (2023) carried out the Effect of Storage and Drying Treatments on Antioxidant Activity and Phenolic Composition of Lemon and Clementine Peel Extracts. The study explained that obtaining polyphenols from horticultural waste is an emerging trend that enables the valorization of resources and the recovery of value-added compounds. However, a pivotal point in the exploitation of these natural extracts is the assessment of their chemical stability. Hence, this study evaluates the effect of temperature storage (20 and -20 °C) and drying methods on the phenolic composition and antioxidant activity of clementine and lemon peel extracts, applying HPLC-DAD-MS, spectrophotometric methods, and chemometric tools. Vacuum-drying treatment at 60 °C proved to be rather suitable for retaining the highest antioxidant activity and the hesperidin, ferulic, and coumaric contents in clementine peel extracts. Lemon extracts showed an increase in phenolic acids after oven-drying at 40 °C, while hesperidin and rutin were sustained better at 60 °C. Hydroethanolic extracts stored for 90 days preserved antioxidant activity and showed an increase in the total phenolic and flavonoid contents in lemon peels, unlike in clementine peels. Additionally, more than 50% of the initial concentration was maintained up to 51 days, highlighting a half-life time of 71 days for hesperidin in lemon peels. Temperature was not significant in the preservation of the polyphenols evaluated, except for in rutin and gallic acid, thus, the extracts could be kept at 20⁰C.

2.3 ANTISTAINING ACTIVITY

1) Enzo A. Palombo., et al. in (2011) performed a Traditional Medicinal Plant Extracts and Natural Products with Activity against Oral Bacteria: Potential Application in the Prevention and Treatment of Oral Diseases. The study characterised that oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable global infectious diseases. Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases. The association between oral diseases and the oral microbiota is well established. Of the more than 750 species of bacteria that inhabit the oral cavity, a number are implicated in oral diseases. The development of dental caries involves acidogenic and aciduric Gram-positive bacteria (mutans streptococci, lactobacilli and actinomycetes). Periodontal diseases have been linked to anaerobic Gram-negative bacteria (*Porphyromonas gingivalis*, *Actinobacillus*, *Prevotella* and *Fusobacterium*). Given the incidence of oral disease, increased resistance by bacteria to antibiotics, adverse effects of some antibacterial agents currently used in dentistry and financial considerations in developing countries, there is a need for alternative prevention and treatment options that are safe, effective and economical. While several agents are commercially available, these chemicals can alter oral microbiota and have undesirable side-effects such as vomiting, diarrhea and tooth staining. Hence, the search for alternative products continues and natural phytochemicals isolated from plants used as traditional medicines are considered as good alternatives. In this review, plant extracts or phytochemicals that inhibit the growth of oral pathogens, reduce the development of biofilms and dental plaque, influence the adhesion of bacteria to surfaces and reduce the symptoms of oral diseases will be discussed further. Clinical studies that have investigated the safety and efficacy of such plant-derived medicines will also be described.

2) Najwa Nasser AL-Jabri., et al. (2018) developed a Chemical composition and antimicrobial potency of locally grown lemon essential oil against selected bacterial strains. The study evaluated the hydro distillation method which was used to isolate and identify essential oils from locally available lemon samples and determine their antimicrobial potency against selected gram positive and negative

bacterial strains using the disk diffusion method. Two locally available and highly consumed lemon fruit samples were used for the isolation of essential oils by hydro distillation using Clevenger type apparatus. The obtained essential oils were analyzed and identified by gas chromatography–mass spectrometry (GC–MS). The essential oils at different concentrations were used to determine antimicrobial potency using one gram (+) *Staphylococcus aureus* (*S. aureus*) and three gram (–) bacteria, *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Proteus vulgaris* (*P. vulgaris*), through the disk diffusion method. Both the essential oils contain twenty-two active compounds with a variation of percentage identified based on GC retention. The main bioactive compounds with high content in Omani sweet lemon essential oil were limonene (84.73%), α -pinene (1.06%), α -terpineol (2.80%), β -myrcene (2.16%), β -pinene (3.36%), terpinen-4-ol (1.16%) and α -terpinolene (2.33%) and several other minor compounds. Similarly, Omani sour lemon essential oil was composed of limonene (53.57%), α -terpineol (14.69%), β -pinene (8.23%), α -pinene (1.84%), β -myrcene (1.51%), α -terpinolene (4.33%), terpinen-4-ol (3.38%), cymene (1.80%), β -bisabolene (1.43%), β -linalool (0.85%) and E-citral (1.08%) with several other minor chemical compounds. Antimicrobial results showed that most concentrations of both essential oils did not give any potential antimicrobial against employing bacterial strains.

3) Bilge Sener., et al. (2019) evaluated a Herbal Extracts Used in Dental Disorders. The study determined that natural products have been recently investigated more thoroughly as promising agents for the prevention of oral diseases, especially plaque-related diseases such as dental caries. Oral diseases, a major health issue in the world, are economically affecting people of developed countries as 10 % of the health expenditure is related to dental care. Different surveys showed that medicinal plant species used by the people for the traditional treatment of dental diseases are inadequately screened for their therapeutic/preventive potential and phytochemical findings. In this review, some herbal extracts commonly used in dental disorders and their bioactivities are outlined.

4) Spartak Yanakiev., et al. (2020) determines the Effects of Cinnamon (*Cinnamomum* spp.) in Dentistry: A Review The study characterized the dental medicine is one of the fields of medicine where the most common pathologies are of bacterial and fungal origins. This review is mainly focused on the antimicrobial effects of cinnamon essential oil (EO), cinnamon extracts, and pure compounds against different oral pathogens and the oral biofilm and the possible effects on soft mouth tissue. Basic information is provided about cinnamon, as is a review of its antimicrobial properties against the most common microorganisms causing dental caries, endodontic and periodontal lesions, and candidiasis. Cinnamon EO, cinnamon extracts, and pure compounds show significant antimicrobial activities against oral pathogens and could be beneficial in caries and periodontal disease prevention, endodontics, and candidiasis treatment.

5) Denga Ramutshatsha-Makhwedzh Avhafunani Mavhungu.,et al. (2022) performed a Activated carbon derived from waste orange and lemon peels for the adsorption of methyl orange and methylene blue dyes from waste water. The study explained adsorbent behaviour in laboratory conditions helps to predict the adsorption process in a large industrial scale. In this study, orange and lemon peels-derived activated carbon (OLPAC) was successfully synthesized and activated using phosphoric acid. Characterization was performed on the OLPAC and the material was used for the removal of methyl orange (MO) and methylene (MB) dyes from wastewater. The results of the scanning electron microscope and N₂ adsorption/desorption examination affirmed that the prepared nanocomposite is permeable, which is an advantage for the efficient removal of contaminants. Optimal conditions for the batch removal process were investigated using a one-factor time approach in different conditions of adsorption (Dye concentration 50–200 mg L⁻¹, pH 2–10, adsorbent mass 0.010–0.8, and contact time 5–180 min. The adsorption isotherm equilibrium data were examined by Langmuir, Freundlich, and Temkin, isotherm model. As shown by the correlation coefficient (R^2), the data were best described by Langmuir isotherms with maximum adsorption capacities of 33 and 38 mg g⁻¹ for methyl orange and methylene blue, respectively. Adsorption kinetic data were described using the pseudo-second-order model which suggests that adsorption of MO and MB was by chemisorption mechanism. The method was applicable to real wastewater samples, with satisfactory removal

percentages of OM and MB (96 and 98 %). The results of this study show that OLPAC is an inexpensive biosorbent that is successfully utilized in removing methyl orange and methylene blue dyes from wastewater.

6) Dolores Bibiana Montero Tomás.,et al. (2023) determines the Effectiveness and abrasiveness of activated charcoal as a whitening agent: A systematic review of in vitro studies. The study described the tooth whitening is currently one of the most requested treatments to change the color of teeth. There are different types of whitening in the dental office and at home. There are also many whitening agents on the market. Nowadays, the public has shown great interest in a new natural compound: activated charcoal. It has an abrasive effect and it is included in toothpastes to whiten teeth quickly and easily. The main objective of the systematic review is to perform a qualitative synthesis of the available literature on the use of activated charcoal-based toothpaste for tooth whitening. An electronic search was carried out in PubMed, Web of Science, and Scopus databases. The search included the terms (charcoal-based OR activated charcoal OR charcoal OR soot) AND (toothpaste OR dentifrices OR bleaching OR oral hygiene OR enamel OR teeth). The results conclude that Out of 208 articles, 11 met the inclusion criteria, the Risk of Bias of the selected studies was determined as medium-high. Regarding the whitening effect, there is a variety of results depending on the study: in some there are no significant differences between the proposed treatments and in others activated charcoal is not the most whitening agent. Regarding the abrasive effect, most studies agree that activated charcoal toothpaste has a higher abrasive potential. They conclude that Toothpastes based on activated charcoal possess a lower whitening effect than other alternatives and can be considered as less safe due to its high abrasive potential.

CHAPTER - 3

OBJECTIVE OF WORK

AIM

The research work aims to prepare the antibacterial, antioxidant and antistaining activity using herbal compound materials for oral hygiene. The source of Phytoconstituents derived from activated charcoal of lemon peels and various used lemon peel powder, cinnamon powder influenced for oral health and to investigate the safety and efficacy of the herbal compound material, focusing anti staining and antibacterial property with antioxidant activity.

OBJECTIVE OF THE WORK

To examine the antibacterial activity of the infected tooth.

To determine the antioxidant activity of phytochemical constituents.

To evaluate the antistaining property of the stained tooth.

To determine the physiochemical properties in order to meet the product quality standards.

CHAPTER 4

EXPERIMENT WORK

4.1 SAMPLE PREPARATION

4.1.1 PREPARATION OF LEMON PEEL

Lemon peel contains calcium, phosphorus, potassium, ascorbic acid and vitamin (A). Lemon peel is derived from the fruit of *Citrus limo*, the common name lemon which was taken as used Lemon (citrus lemon) from various source was washed on the flowing water and peeled them. Lemon peel where sliced and dried under the sunlight for 12hrs. The dried lemon was subjected for grounded at moderate rotation in a regular blender, and the powdered material was then carefully collected and stored at room temperature in an air tight bottle.

4.1.2 PREPARATION OF ACTIVATED CHARCOAL

The activated carbon was synthesized following a modified method (Fernandez *et al.*, 2014) b lemon skins were collected from a fresh market, thoroughly washed with deionized water and separately dried in the oven at 110°C for 24hrs. The dried were then pulverized into small particles sizes ranging from 200-400µm equal amounts of lemon skins were mixed and properly homogenized before impregnation with an equal volume of 85% H₃PO₄. The excess liquid was removed and the material was dried for 24hrs in the oven at 110°C. Therefore, the carbon was placed in a furnace at 600°C for 3hrs. The chemically activated carbon was cleansed using distilled water and dried at 110°C. The dried lemon was subjected for grounded at moderate rotation in a regular blender, and the powdered material was then carefully collected and stored at room temperature in an air tight bottle.

4.1.3 PREPARATION OF CINNAMON

Dried cinnamon bark was taken from a kitchen store. The cinnamon bark was prepared using a kitchen based blender for size reduction and this powder is called general/crude powder of cinnamon. The powder color of cinnamon ranges from yellowish brown (Ceylon), reddish brown (Indonesian and Vietnamese), darker brown (Chinese). The powdered material was then carefully collected and stored at room temperature in an air tight bottle.

4.2 ANTIBACTERIAL ACTIVITY

4.2.1 SAMPLE

L+Ci - Lemon peel with cinnamon

AL + Ci - Activated charcoal with cinnamon

4.2.2 MATERIALS REQUIRED

Muller Hinton agar (MHA) plates Muller Hinton broth (MHB)
Micropipette tips Microbial loop Sterile cotton swab 0.5 Mc Farland
standard Cork borer Antibiotics – Ciprofloxacin (5mcg). The test
pathogens are Escherichia coli ATCC 25922, Staphylococcus aureus
ATCC 29213 and Klebsiella pneumoniae ATCC 700603

4.2.4 SAMPLE PREPARATION

Given samples were coded as L+Ci and AL+Ci and dissolve the test
samples with 10% DMSO at 1 mg/mL concentration and prepare the
stock solution. Make further dilutions from stock as working
concentration

(500 µg/mL -31.25 µg/mL)(500, 250, 125, 62.5 and 31.25 µg/mL)

4.2.5 PREPARATION OF BACTERIAL INOCULUM

A loopful of test pathogens were inoculated in Muller Hinton broth MHB respectively and adjusted to 0.5 McFarland standard.

4.2.6. PROCEDURE

The antibacterial activity was analysed by well diffusion method. Using sterile cotton swab, the test bacterial inoculum was seeded on the Muller Hinton Agar plates. The wells were made using cork borer. 100 µl of samples were pipetted out in respective wells and incubated the plates at 37 °C for 24 hrs. After incubation, zone of inhibition (ZOI) around the wells were measured in diameter (mm) and noted.

4.3. ANTIOXIDANT ACTIVITY

4.3.1. HYDROGEN PEROXIDE METHOD

4.3.1.2 SAMPLE PREPARATION

The compounds are,

Compound (1) – Dried lemon peel with charcoal (LC)

Compound (2)- Dried lemon peel powder (LP)

Compound (3)- Dried lemon peel powder with cinnamon powder (L+Ci)

4.3.1.2.1 SAMPLE PREPARATION WITH METHANOL

Weighed accurately 10mg of compound (1) and dissolved with 10ml of methanol and filtered. Weighed accurately of 10mg of Compound (2) and dissolved with 10ml of methanol and filtered. Weighed accurately of 10mg of compound (3) and dissolved with 10ml of methanol and filtered.

4.3.1.2.2 SAMPLE PREPARATION WITH ACETONE

Weighed accurately of 10mg of compound (1) and dissolved with 10ml of acetone and filtered. Weighed accurately of 10mg of compound (2), and dissolved with 10ml of acetone and filtered. Weighed accurately of 10mg of compound (3) and dissolved with 10ml of acetone and filtered.

4.3.1.2.3. SAMPLE PREPARATION WITH WATER

Weighed accurately 10mg of compound (1) and dissolved with 10ml of water and filtered. Weighed accurately of 10mg of compound (2) and dissolved with 10ml of water and filtered. Weighed accurately of 10mg of compound (3) and dissolved with 10ml of water and filtered.

4.3.1.3 PROCEDURE

The compounds (1,2,3) were prepared by using various solvents methanol, acetone and water. The methanol, acetone and water stock solution with varying quantities were added to 3.8ml of 0.1M phosphate buffer solution (PH 7.4) and then mixed with 0.2ml of hydrogen peroxide solution. The absorbance of the reaction mixture was measured at 230nm after 10min. The mixture without sample was used as blank. Ascorbic acid was used as standard.

The percentage inhibition of hydrogen peroxide is calculated by using the formula.

Percentage inhibition = $(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 10$

4.4. ANTISTAINING ACTIVITY

4.4.1. SAMPLE

Compound material (1) - Dried Lemon Peel Powder

Compound material (2) - Dried Lemon Peel Charcoal Powder

Compound material (3) - Dried Lemon Peel powder + cinnamon Powder

LC – DRIED LEMON PEEL CHARCOAL

LP - DRIED LEMON PEEL POWDER

L+ Ci – DRIED LEMON PEEL + CINNAMON POWDER

4.4.2. PROCEDURE FOR DYE ADSORPTION

Weighed accurately 10mg of methylene blue and diluted with 10ml of water. Shake it until it's completely dissolve. This 10mg /10ml solution was stock for control. Weighed accurately of 10mg of each compound material (1 ,2 and 3) was measured and diluted with 10ml of water and shake it well until its completely dissolve. This10 mg/ml solution was stock for control. Varying concentration (10 to 100 µg/ml) of compound material was prepared.

4.4.3. METHYLENE BLUE CONCENTRATION

MB = +0.5 mcg/ml

Compound -1 (10µg/ml-100 µg/ml)

Compound -2 (10 µg/ml -100 µg/ml)

Compound -3 (10 µg/ml -100 µgml)

4.4.4 TOOTH ANTISTAINING ACTIVITY

4.4.4.1 SAMPLE PREPARATION

The compound solution is prepared by Weighed accurately 5mg of compound material (1) and dissolve it with the 5ml of water in a test tube. Separately weighted 5mg of compound material (2) and dissolve it with the 5ml of water in a test tube. Again, separately weighed 5mg of compound material (3) and dissolve it with the 5ml of water in a test tube. Each compound material (1,2,3.) was observed in a China dish.

The compound materials are:

Compound material (1) – Dried lemon peel charcoal. (LC)

Compound material (2) – Dried lemon peel powder. (LP)

Compound material (3) – Dried lemon peel powder+ cinnamon powder (L+Ci)

4.4.4.2. PROCEDURE

The first method of tooth antistaining is Soaking. The three different stained tooth were soaked in the compound solution (1), (2) and (3) for five minutes.



Figure (a) - Compound (1)



Figure (b) – Compound (2)



Figure (c) – Compound (3)

Figure 4.1 (a) (b)(c) Picture shown the soaking process of compound (1)(2)(3)

The second Method of tooth antistaining is Mechanical brushing. After soaking, brush the tooth in the compound solution (1) (2) (3) for two minutes.

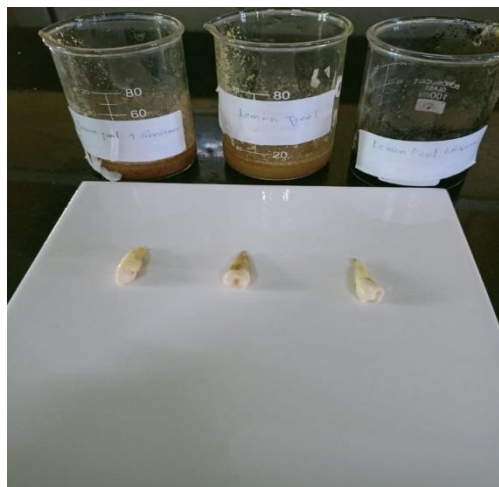


Figure 4.2 Picture shown Mechanical Brushing Of compound (1),(2),(3).

4.5 PHYSIOCHEMICAL PROPERTIES OF HERBAL CONSTITUENTS

4.5.1 SOLUBILITY

Table 4.1 Comparing solubility test of (LC,LP,L+Ci)

S.no	Compound	Water	Methanol	Acetone
1.	Compound (1) (LC)	Not Soluble	Soluble	Not Soluble
2.	Compound (2) (LP)	Not Soluble	Soluble	Not Soluble
3.	Compound (3) (L+Ci)	Not Soluble	Soluble	Not Soluble

4.5.2. FTIR

FTIR is a Fourier-transform infrared spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid, or gas. An FTIR spectrometer simultaneously collects high-resolution spectral data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time.

The samples of FTIR are:

Compound (1) - Dried Lemon Peel Charcoal

Compound (2) - Dried Lemon Peel Powder

Compound (3) - Dried Lemon Peel Charcoal + Cinnamon Powder

4.5.2.1 Compound 1- Dried Lemon Peel Charcoal

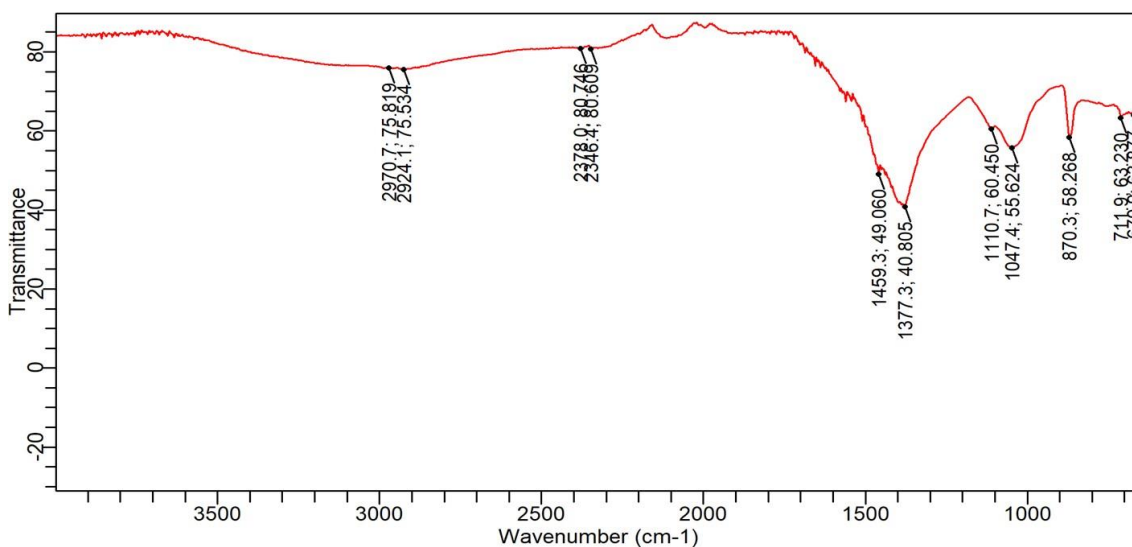


Figure 4.3 Graph shown FTIR of LC

Table 4.2 FTIR table for LC

Peak Number	Wavenumber (cm ⁻¹)	Intensity	Functional groups
1	833.06001	56.40784	Alkenes 780-850 C=C med. Amines 660-900 N-H var Arenes 690-900 C-H str.
2	892.69741	52.23991	Alkenes 880-995 str. Arenes 690-900 var.
3	1011.97222	30.91834	Amines 1000-1250 med Carboxylic acids and derivatives 1000-1300 str. Alcohol and phenols 970-1250 med.
4	1049.24560	35.10210	Amines 1000-1250 med. Alcohol and phenols 970-1250 str. Carboxylic acids and derivatives 1000-1300 str.
5	1097.70099	40.77928	Alcohols and phenols 970-1250

			str. Amines 1000-1250 med Carboxylic acid and derivatives 1000-1300 str.
6	1146.15638	51.26685	Arenes 1600-1500 med.wk Alcohols and phenols 970-1250 str Amines 1000-1250 str.
7	1220.70314	47.11829	Alcohols and phenols 970-1250 str. Amines 1000-1250 med Carboxylic acids and derivatives 1210-1320 med.str.
8	1319.47759	56.79493	Carboxylic acid derivatives 1210- 1320 med.str.
9	1366.06932	57.29242	Alcohols and phenols 1330-1480 med. Aldehydes and ketones 1350- 1360 str.
10	1399.61536	58.30137	Alkanes 1370-1390 med. Alcohols and phenols 1380-1430 med.
11	1638.16498	56.47048	Carboxylic acids and derivatives 1590-1650 med. Amines 1550-1650 med.str
12	1656.80167	58.41519	Carboxylic acids and derivatives 1630-1695
13	1720.16641	52.87988	Aldehydes and ketones 1720- 1740 str.
14	1912.12431	91.07994	Alkenes 1900-2000 str.
15	1995.98941	93.41335	Alkenes 1900-2000 str.
16	2115.26422	92.95730	Nitrites 2100-2270 med.
17	2855.14078	72.86458	Alkanes 2850-3000 str.

4 5.2.2 Compound 2- Dried Lemon Peel

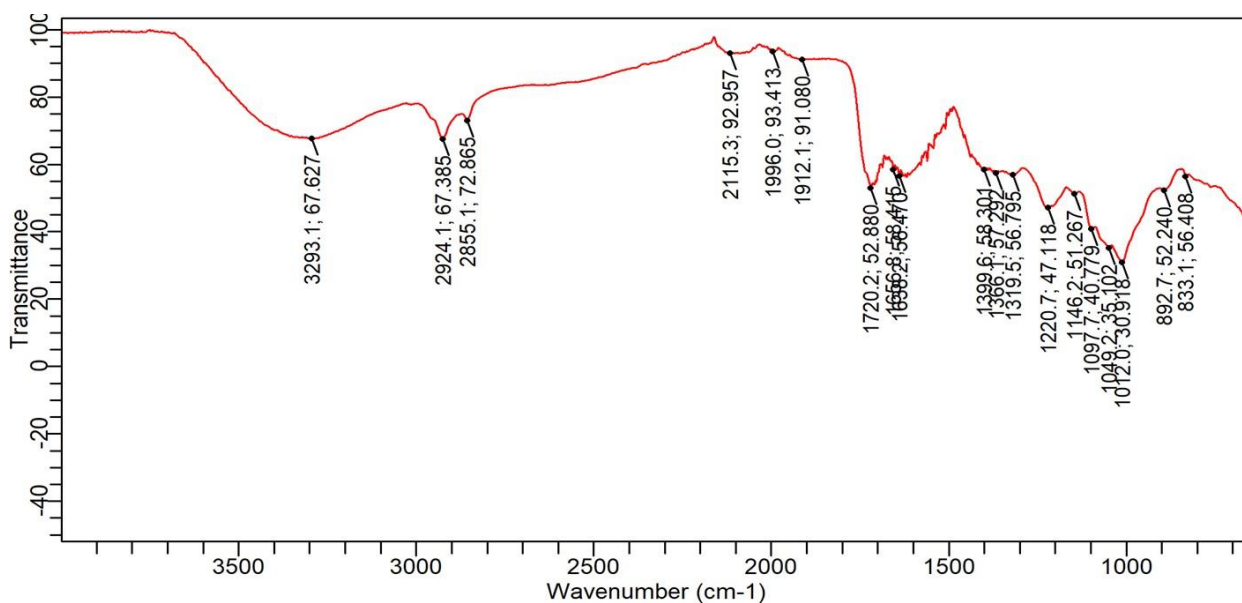


Figure 4.4 Graph shown FTIR of LP

Table 4.3 FTIR table for LP

Peak Number	Wavenumber (cm ⁻¹)	Intensity	Functional groups
1	833.06001	56.40784	Alkenes 780-850 C=C med. Amines 660-900 N-H var Arenes 690-900 C-H str.
2	892.69741	52.23991	Alkenes 880-995 str. Arenes 690-900 var.
3	1011.97222	30.91834	Amines 1000-1250 med Carboxylic acids and derivatives 1000-1300 str. Alcohol and phenols 970-1250 med.
4	1049.24560	35.10210	Amines 1000-1250 med. Alcohol and phenols 970-1250 str. Carboxylic acids and derivatives

			1000-1300 str.
5	1097.70099	40.77928	Alcohols and phenols 970-1250 str. Amines 1000-1250 med Carboxylic acid and derivatives 1000-1300 str.
6	1146.15638	51.26685	Arenes 1600-1500 med.wk Alcohols and phenols 970-1250 str. Amines 1000-1250 str.
7	1220.70314	47.11829	Alcohols and phenols 970-1250 str. Amines 1000-1250 med Carboxylic acids and derivatives 1210-1320 med.str.
8	1319.47759	56.79493	Carboxylic acid derivatives 1210-1320 med.str.
9	1366.06932	57.29242	Alcohols and phenols 1330-1480 med. Aldehydes and ketones 1350-1360 str.
10	1399.61536	58.30137	Alkanes 1370-1390 med. Alcohols and phenols 1380-1430 med.
11	1638.16498	56.47048	Carboxylic acids and derivatives 1590-1650 med. Amines 1550-1650 med.str
12	1656.80167	58.41519	Carboxylic acids and derivatives 1630-1695
13	1720.16641	52.87988	Aldehydes and ketones 1720-1740 str.
14	1912.12431	91.07994	Alkenes 1900-2000 str.
15	1995.98941	93.41335	Alkenes 1900-2000 str.
16	2115.26422	92.95730	Nitrites 2100-2270 med.
17	2855.14078	72.86458	Alkanes 2850-3000 str.

4.5.2.3 Compound 3-Dried Lemon Peel Charcoal+Cinnamon.

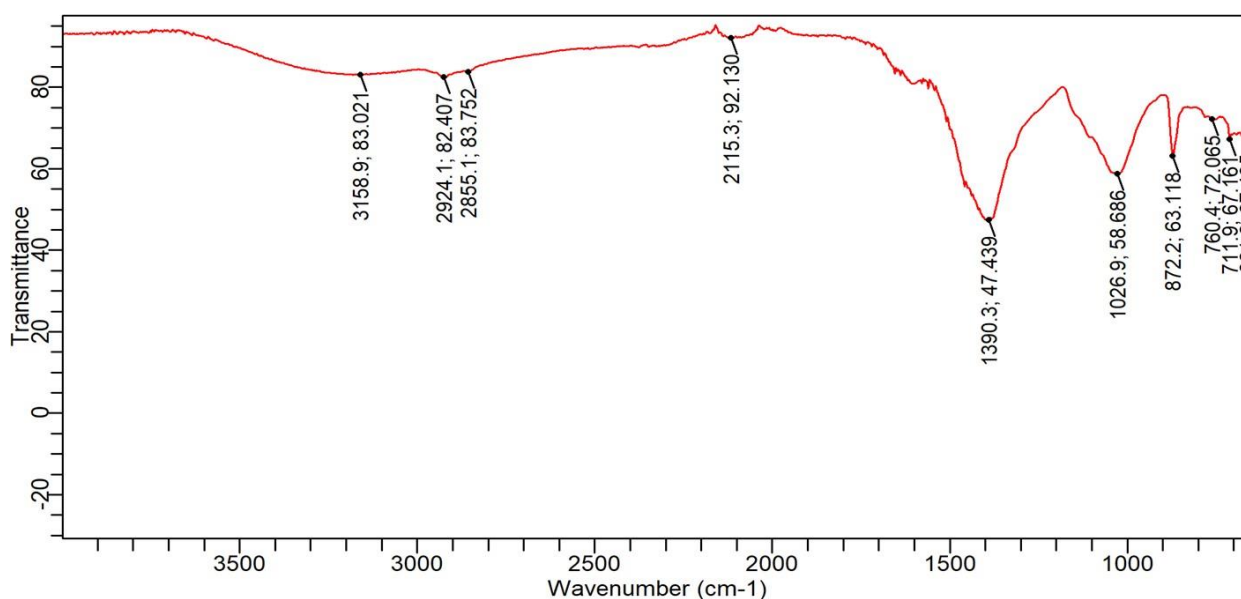


Figure 4.5 Graph shown FTIR of LC+Ci

Table 4.4 FTIR table for LC+Ci

Peak Number	Wavenumber (cm ⁻¹)	Intensity	Functional groups
1	661.60247	67.49504	Alkynes 600-700 str. Alcohol and phenols 650-770 var.wk Amines 660-900 var.
2	711.92153	67.16075	Alkenes 675-730 med. Alkynes 600-700 str. Alcohol and phenols 650-770 var-wk Amines 660-900 var Arenes 690-900 str-med
3	760.37692	72.06473	Alcohol and phenols 650-770 var-wk Amines 660-900 var Arenes 690-900 str-med

4	872.19705	63.11815	Amines 660-900 var Arenes 690-900 str-med
5	1026.88157	58.68552	Alcohols and phenols 970-1250 med Amines 1000-1250 med Carboxylic acids and derivatives 1000-1300 str
6	1390.29701	47.43852	Alkanes 1350-1470 med Alkanes 1370-1390 med Alcohols and phenols 1330- 1430 med
7	2115.26422	92.13025	Alkynes 2100-2250 var Isocyanates, isothiocyanates,
8	2855.14078	83.75237	Carboxylic acids and derivatives 2500-3300(acids)overlaps C-H str
9	2924.09653	82.40700	Carboxylic acids and derivatives 2500-3300(acids)overlaps C-H str
10	3158.91882	83.02132	Carboxylic acids and derivatives 2500-3300(acids)overlaps C-H str

CHAPTER- 5

5. RESULTS AND DISCUSSION

5.1 ANTIBACTERIAL ACTIVITY

5.1.1 OBSERVATION:

Table 5.1 Antibacterial efficacy of L+C, AL+Ci

Sample ID	Zone of Inhibition in diameter (mm)					
	500 µg/ml(1)	250 µg/ml(2)	125 µg/ml(3)	62.5 µg/ml(4)	31.25 µg/ml(5)	Control (C)
<i>E. coli</i> ATCC 25922						
L+Ci	-	-	-	-	-	16
AL+Ci	-	-	-	-	-	16
<i>Klebsiella pneumoniae</i> ATCC 700603						
L+Ci	-	-	-	-	-	16
AL+Ci	-	-	-	-	-	16
<i>Staphylococcus aureus</i> ATCC 29213						
L+Ci	16.5	16	15	-	-	16
AL+Ci	-	-	-	-	-	16

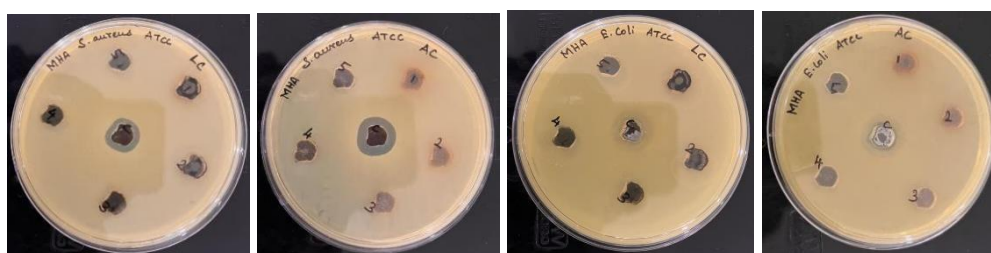


Figure 5.1 antibacterial activity test

5.1.2 DISCUSSION

Table 5.2 Antibacterial activity of L+Ci and AL+Ci

S.NO	SAMPLE	PATHOGEN	RESULT
1.	L+Ci	<i>Staphylococcus aureus</i>	15mm of zone of inhibition against Gram positive (<i>Staphylococcus aureus</i>)
2.	AL+Ci	<i>E. coli</i> <i>Klebsiella pneumonia</i> <i>Staphylococcus aureus</i>	-

The sample L+Ci showed 15 mm of zone of inhibition against Gram positive *Staphylococcus aureus* at concentration 500µg/ml, 250µg/ml and 125µg/ml. Due to inhibition of antibacterial activity. No result was observed in the sample AL+ Ci. This may have occurred due to the changes in physiological properties of sample materials.

5.2 ANTIOXIDANT ACTIVITY

5.2.1 DRIED LEMON PEEL CHARCOAL WITH METHANOL

Table5.3 Antioxidant activity of LC with methanol

S.NO	Concentration ($\mu\text{g/ml}$)	Inhibition Of Sample Absorbance(%)
1.	50	92.6
2	100	97.29
3	200	95.9
4	300	90.77
5	400	90.03
		$\text{IC}_{50}=93.31$

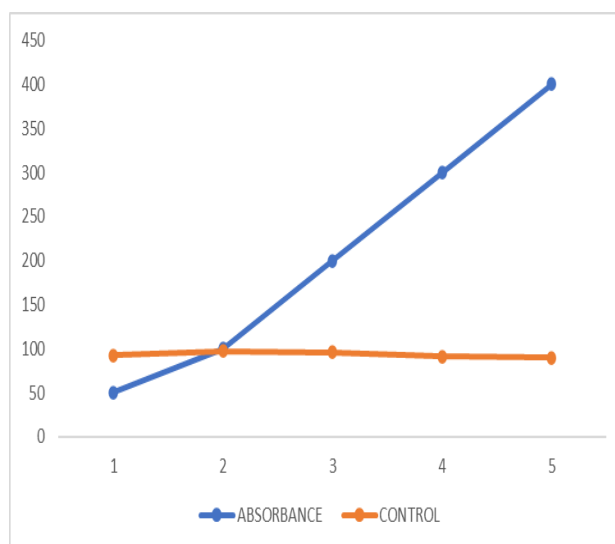


Figure 5.2 Graph shown Antioxidant activity of LC with methanol

The sample denotes the dried lemon peel charcoal absorbance and Control denotes the concentration. In this, there are 5 concentration ranges from 50, 100, 200, 300, 400 ($\mu\text{g/ml}$). The inhibition of sample absorbance are ranges from 92.6, 97.29, 95.9, 90.77, 90.0(%). The above each concentration and their absorbance showed the **moderate activity**.

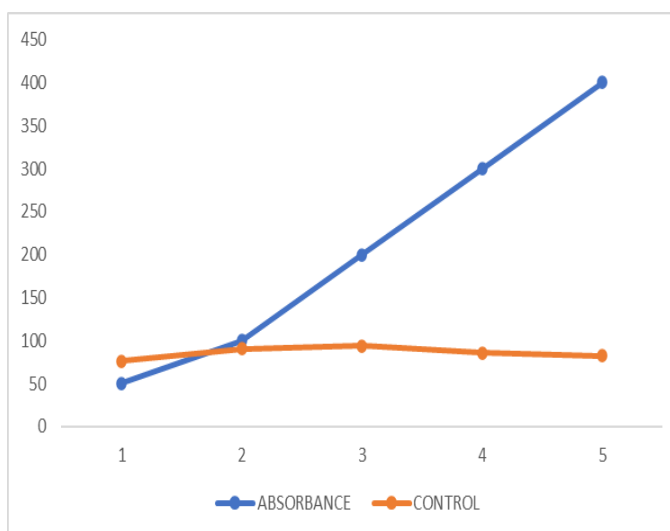
5.2.2 DRIED LEMON PEEL POWDER WITH METHANOL

Table 5.4 Antioxidant activity of LP with methanol

S.NO	Concentration (µg/ml)	Inhibition Of Sample Absorbance (%)
1	50	75.97
2	100	90.41
3	200	93.71
4	300	85.94
5	400	82.11
		IC ₅₀ = 85.63

Figure 5.3 Graph shown Antioxidant activity of LP with methanol

The series 1 denotes the dried lemon peel powder absorbance and Series 2



denotes the concentration In this, there are 5 concentration ranges from 50, 100, 200, 300, 400 (µg/ml). The inhibition of sample absorbance are ranges from 75.97,90.41, 93.71, 85.94, 82.11(%). The above each concentration and their absorbance showed the **moderate activity**.

5.2.3 DRIED LEMON PEEL + CINNAMON WITH METHANOL

Table 5.5 Antioxidant activity of L+Ci with methanol

S.NO	Concentration ($\mu\text{g/ml}$)	Inhibition Of Sample Absorbance (%)
1	50	30.54
2	100	74.39
3	200	84.29
4	300	67.02
5	400	42.34
		$\text{IC}_{50} = 59.71$

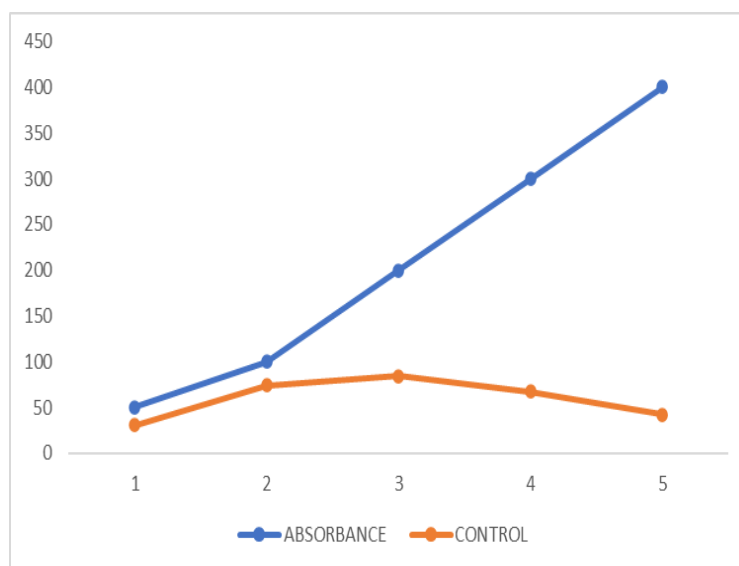


Figure 5.4 Graph shown antioxidant activity of L+Ci with methanol

The sample denotes the dried lemon peel with cinnamon absorbance and Control denotes the concentration in this, there are 5 concentration ranges from 50, 100, 200, 300, 400 ($\mu\text{g/ml}$). The inhibition of sample absorbance are ranges from 30.54, 74.39, 84.29, 67.02, 42.34(%). The concentration (50 $\mu\text{g/ml}$, 400 $\mu\text{g/ml}$) showed good antioxidant activity. The remaining concentration showed the moderate activity.

5.2.4 DRIED LEMON PEEL CHARCOAL WITH ACETONE

Table5.6 Antioxidant activity of LC with acetone

S.NO	Concentration ($\mu\text{g/ml}$)	Inhibition Of Sample Absorbance (%)
1	50	151.19
2	100	277.71
3	200	100
4	300	92.475
5	400	102.42
		$\text{IC}_{50} = 144.75$

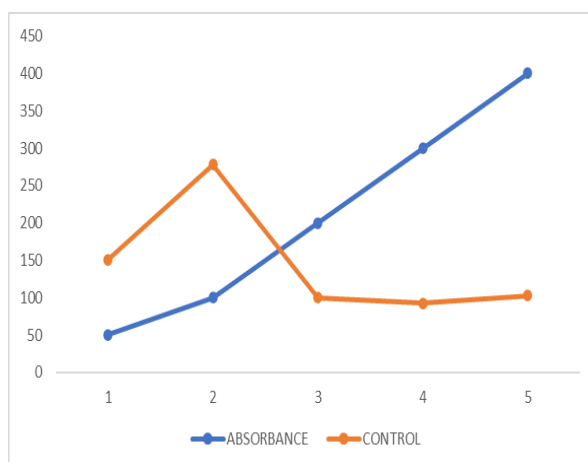


Figure 5.5 Graph shown Antioxidant activity of LC with acetone

The sample denotes the dried lemon peel charcoal absorbance and control denotes the concentration. In this, there are concentration ranges from 50, 100, 200, 300, 400 ($\mu\text{g/ml}$). The inhibition of sample absorbance are ranges from 151.19, 277.71, 100, 92.475, 102.42(%). The above each concentration and their absorbance showed the moderate activity.

5.2.5 DRIED LEMON PEEL POWDER WITH ACETONE

Table 5.7 Antioxidant activity of LP with acetone.

S.NO	Concentration ($\mu\text{g/ml}$)	Inhibition Of Sample Absorbance (%)
1	50	10
2	100	113.37
3	200	107.92
4	300	107.52
5	400	200
		$\text{IC}_{50} = 107.76$

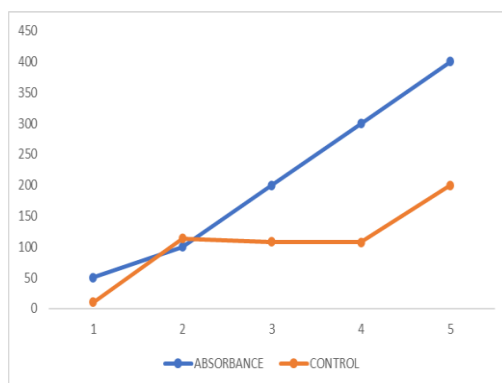


Figure 5.6 Graph shown Antioxidant activity of LP with acetone

The sample denotes the dried lemon peel powder absorbance and Control denotes the concentration. In this, there are 5 concentration ranges from 50, 100, 200, 300, 400 ($\mu\text{g/ml}$). The inhibition of sample absorbance are ranges from 10, 113.37, 107.92, 107.52, 200. The First concentrations 50 $\mu\text{g/ml}$ showed good antioxidant activity. The remaining concentration showed the moderate activity.

5.2.6 DRIED LEMON PEEL + CINNAMON WITH ACETONE

Table 5.8 Antioxidant activity of LP +Ci with acetone

S.NO	Concentration ($\mu\text{g/ml}$)	Inhibition Of Sample Absorbance (%)
1	50	201.61
2	100	277.71
3	200	205.31
4	300	200
5	400	200
		IC₅₀ = 216.92

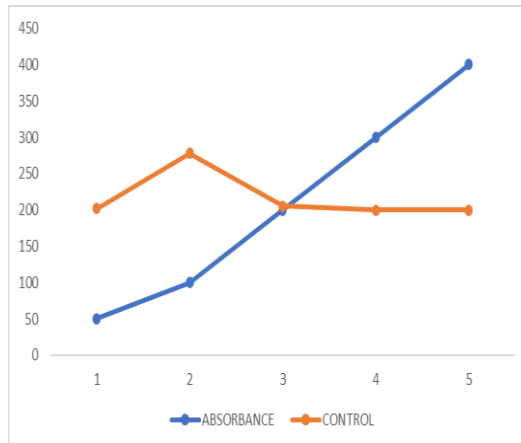


Figure 5.7 Graph shown Antioxidant activity of L+Ci with acetone

The sample denotes the dried lemon peel with cinnamon absorbance and Control denotes the concentration. In this, there are 5 concentration ranges from 50, 100, 200, 300, 400 ($\mu\text{g/ml}$). The inhibition of sample absorbance are ranges from 202.62, 277.71, 205.31, 200, 200(%). The above each concentration and their absorbance showed the moderate activity.

5.2.7 DRIED LEMON PEEL CHARCOAL WITH WATER

Table 5.9 Antioxidant activity of LC with water

S.NO	Concentration ($\mu\text{g/ml}$)	Inhibition Of Sample Absorbance (%)
1	50	98.29
2	100	94.17
3	200	99.23
4	300	93.62
5	400	87.34
		IC₅₀ = 94.53

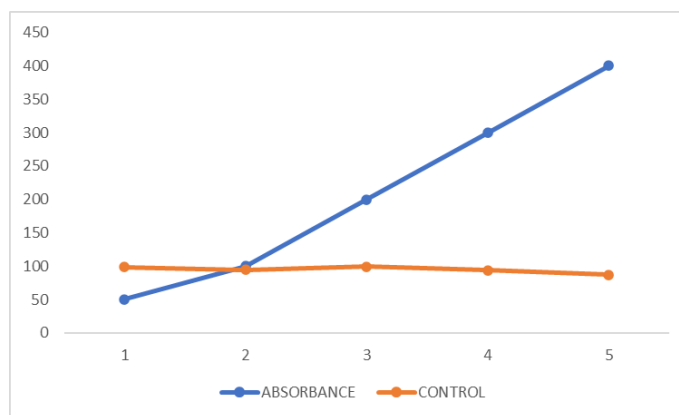


Figure 5.8 Graph shown Antioxidant activity of LC with water.

The sample denotes the dried lemon peel charcoal absorbance and Control denotes the concentration. In this, there are 5 concentration ranges from 50, 100, 200, 400 ($\mu\text{g/ml}$). The inhibition of sample absorbance are ranges from 98.29, 94.17, 99.23, 93.62, 87.34(%). The above each concentration and their absorbance showed the moderate activity.

5.2.8 DRIED LEMON PEEL POWDER WITH WATER

Table 5.10 Antioxidant activity of LP with water

S.NO	Concentration ($\mu\text{g/ml}$)	Inhibition Of Sample Absorbance (%)
1	50	97.81
2	100	94.80
3	200	96.31
4	300	94.57
5	400	88.77
		IC₅₀ = 94.45

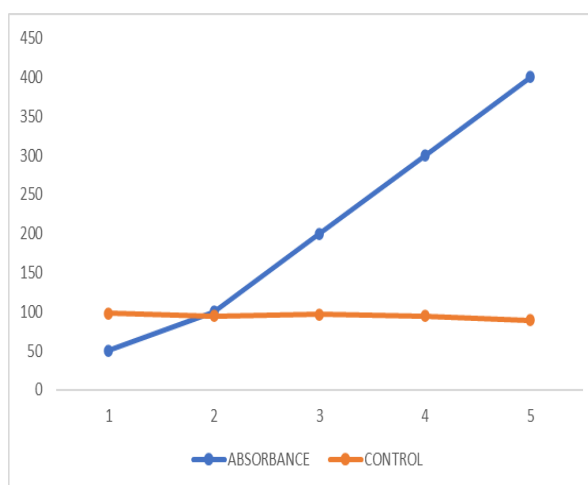


Figure 5.9 Graph shown Antioxidant activity of LP with Water

The sample denotes the dried lemon peel powder absorbance and Control denotes the concentration. In this, there are 5 concentration ranges from 50, 100, 200, 400 ($\mu\text{g/ml}$). The inhibition of sample absorbance are ranges from 93.81, 94.80, 96.31, 94.57, 88.77(%). The above each concentration and their absorbance showed the **moderate activity**.

5.2.9 DRIED LEMON PEEL + CINNAMON WITH WATER

Table 5.11 Antioxidant activity of L+Ci with water

S.NO	Concentration ($\mu\text{g/ml}$)	Inhibition Of Sample Absorbance (%)
1	50	35.31
2	100	78.26
3	200	78.93
4	300	75.02
5	400	26.82
		$\text{IC}_{50} = 58.87$

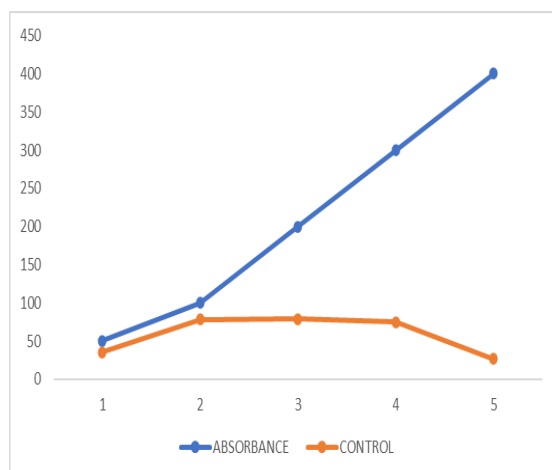


Figure 5.10 Graph shown Antioxidant activity of L+Ci with Water

The sample denotes the dried lemon peel with cinnamon absorbance and Control denotes the concentration. In this, there are 5 concentration ranges from 50, 100, 200, 400 ($\mu\text{g/ml}$). The inhibition of sample absorbance are ranges from 35.31, 78.26, 78.93, 75.02, 26.82(%). The first concentration **50 $\mu\text{g/ml}$** and the last concentration **400 $\mu\text{g/ml}$** showed **good antioxidant activity**.

5.2.10 DISCUSSION

Table 5.12Antioxidant activity of compound material
LC,LP and L+Ci

S.NO	COMPOUND MATERIAL	SOLVENT	RESULT
1	LC	Methanol	Moderate antioxidant activity.
		Acetone	Moderate antioxidant activity.
		Water	Moderate antioxidant activity.
2	LP	Methanol	Moderate antioxidant activity.
		Acetone	Good antioxidant activity.
		Water	Moderate antioxidant activity.
3	L+Ci	Methanol	Moderate antioxidant activity.
		Acetone	Moderate antioxidant activity.
		Water	Good antioxidant activity.

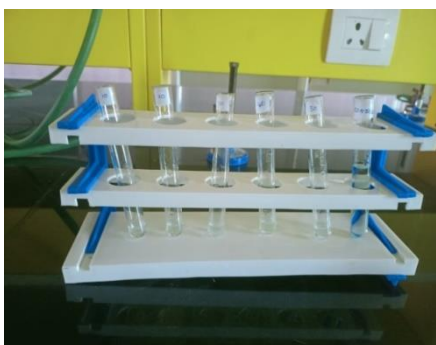
Moderate antioxidant activity was found with solvent methanol, acetone and water in compound material LC. Moderate activity may have occurred due to the change in the compound material LC's physiochemical properties. Good antioxidant activity was found in acetone and moderate antioxidant activity was found in methanol and water in compound material LP. Moderate activity may have occurred due to the change in the compound material LP's physiochemical properties. Good antioxidant activity was found in solvent water and moderate antioxidant activity was found in solvent methanol and acetone in compound material L+Ci. Moderate activity may have occurred due to the change in the compound material L+ci physiochemical properties.

5.3 ANTISTAINING ACTIVITY WITH DYE METHOD

5.3.1 DRIED LEMON PEEL POWDER

Table 5.13 Antistaining activity of methyleneblue concentration with compound (1)

Methylene Blue Concentration ($\mu\text{g/ml}$)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Compound-1 ($\mu\text{g/ml}$)	10	20	30	40	50	60	70	80	90	100
Time taken to decolourise (sec)	0.22	0.75	2.13	1.94	1.06	1.91	2.18	1.91	1.55	1.32



(a) 10 $\mu\text{g/ml}$ -50 $\mu\text{g/ml}$



(b) 60 $\mu\text{g/ml}$ -100 $\mu\text{g/ml}$

Figure 5.11 (a),(b) Picture shown the control methylene blue without compound (1)



(c) 10 $\mu\text{g/ml}$ -50 $\mu\text{g/ml}$



(d) 60 $\mu\text{g/ml}$ -100 $\mu\text{g/ml}$

Figure 5.12 (c),(d) Picture shown the control methylene blue with range of concentration compound (1)

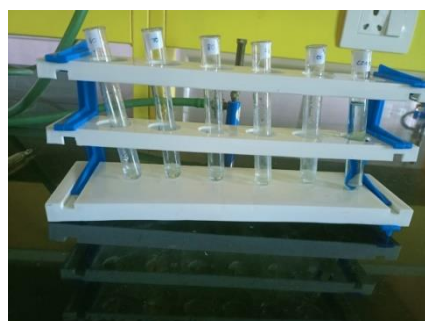
5.3.2 DRIED LEMON PEEL CHARCOAL POWDER

Table 5.14 Antistaining activity of methylene blue concentration with compound (2)

Methylene Blue Concentration ($\mu\text{g/ml}$)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Compound-2 ($\mu\text{g/ml}$)	10	20	30	40	50	60	70	80	90	100
Time taken to decolourise (sec)	2.40	1.62	2.92	1.40	1.48	1.45	1.31	2.01	2.14	2.34

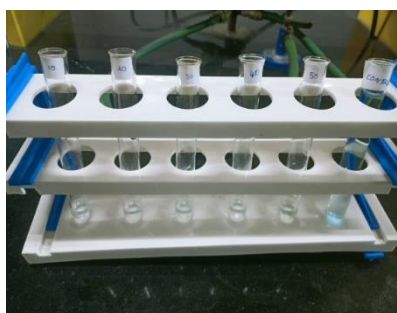


(a) 10 $\mu\text{g/ml}$ -50 $\mu\text{g/ml}$

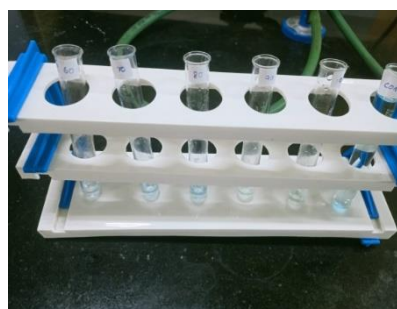


(b) 60 $\mu\text{g/ml}$ -100 $\mu\text{g/ml}$

Figure 5.13 (a),(b) Picture shown the control methylene blue without compound (2)



(c) 10 $\mu\text{g/ml}$ -50 $\mu\text{g/ml}$



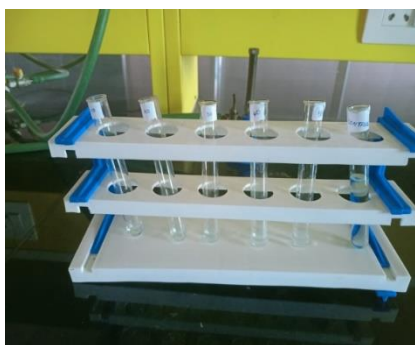
(d) 60 $\mu\text{g/ml}$ -100 $\mu\text{g/ml}$

Figure 5.14 (c),(d) Picture shown the control methyleneblue with range of concentration compound (2)

5.3.3 DRIED LEMON PELL POWDER + CINNAMON POWDER

Table 5.15. Antistaining activity of methylene blue concentration with compound (3)

Methylene Blue Concentration ($\mu\text{g/ml}$)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Compound-3 ($\mu\text{g/ml}$)	10	20	30	40	50	60	70	80	90	100
Time taken to decolourise (sec)	2.58	1.93	1.45	1.65	1.47	1.57	1.53	1.81	1.96	1.59



(a) 10 $\mu\text{g/ml}$ -50 $\mu\text{g/ml}$



(b) 60 $\mu\text{g/ml}$ -100 $\mu\text{g/ml}$

Figure 5.15 (a),(b) Picture the shown the control methylene blue without compound (3)



(c) 60 $\mu\text{g/ml}$ -100 $\mu\text{g/ml}$



(d) 60 $\mu\text{g/ml}$ -100 $\mu\text{g/ml}$

Figure 5.16 (c),(d) Picture shown the control methyleneblue with range of concentration compound (3)

5.3.4 DISCUSSION

Table 5.16 Antistaining activity of compound material (LP,LC,L+Ci)

S.NO	Compound Material	Methylene Blue Concentration	Result
1	LP (10-100µg/ml)	0.5µg/ml	Good
2	LC (10-100µg/ml)	0.5µg/ml	Good
3	L+Ci(10-100µg/ml)	0.5µg/ml	Good

Good anti-staining activity was observed in methylene blue at concentration 0.5µg/ml with compound material LP, LC, L+Ci due to their adsorption property.

5.3.3.1 ANTISTAINING ACTIVITY WITH TOOTH MOULD



Figure 5.17 Picture shown Before Antistaining activity



Figure 5.18 Picture shown After Antistaining activity

5.3.3.2 Result

Using compound material (1) (2) and (3) for various tooth and their stain was removed accordingly by using two methods.

5.3.3.3 DISCUSSION

Table 5.17 Tooth Anstaining activity of compound material (LP,LC,L+Ci)

S.NO	Compound Material	Result
1	LP	Moderate
2	LC	Moderate
3	L+ Ci	Moderate

Tooth anti- staining of compound material 1,2 and 3 gives a moderate result due to usage of water solvent instead of alcohol.

CHAPTER-6

6. SUMMARY AND CONCLUSION

6.1 ANTIBACTERIAL ACTIVITY

Table 6.1 Antibacterial activity of sample (L+Ci ,AL+Ci)

S.NO	SAMPLE	PATHOGEN	RESULT
1.	L+ Ci	<i>Staphylococcus aureus</i>	15mm of of zone of inhibition against Gram positive (<i>Staphylococcus aureus</i>)
2.	AL+ Ci	<i>E. coli</i> <i>Klebsiella pneumonia</i> <i>Staphylococcus aureus</i>	-

The sample LC showed 15 mm of zone of inhibition against Gram positive *Staphylococcus aureus* at concentration 500µg/ml, 250µg/ml and 125µg/ml. Due to inhibition of antibacterial activity. No result was observed in the sample AL+ Ci. This may have occurred due to the changes in physiological properties of sample materials.

6.2 ANTIOXIDANT ACTIVITY

Table 6.2 Antioxidant activity of compound materials (LC,LP,L+Ci)

S.NO	COMPOUND MATERIAL	SOLVENT	RESULT
1	LC	Methanol	Moderate antioxidant activity.
		Acetone	Moderate antioxidant activity.
		Water	Moderate antioxidant activity.
2	LP	Methanol	Moderate antioxidant activity.
		Acetone	Good antioxidant activity.
		Water	Moderate antioxidant activity.
3	L+ Ci	Methanol	Moderate antioxidant activity.
		Acetone	Moderate antioxidant activity.
		Water	Good antioxidant activity.

Moderate antioxidant activity was found with solvent methanol, acetone and water in compound material LC. Moderate activity may have occurred due to the change in the compound material LC's physiochemical properties. Good antioxidant activity was found in acetone and moderate antioxidant activity was found in methanol and water in compound material LP. Moderate activity may have occurred due to the change in the compound material LP's physiochemical properties. Good antioxidant activity was found in solvent water and moderate

antioxidant activity was found in solvent methanol and acetone in compound material L+ Ci. Moderate activity may have occurred due to the change in the compound material's (L+ ci) physiochemical properties.

6.3 ANTISTAINING ACTIVITY

Table 6.3 Antistaining activity of compound material (LP,LC,L+Ci)

S.NO	Compound Material	Methylene Blue Concentration	Result
1	LP (10-100µg/ml)	0.5µg/ml	Good
2	LC (10-100µg/ml)	0.5µg/ml	Good
3	L+Ci (10-100µg/ml)	0.5µg/ml	Good

Good anti-staining activity was observed in methylene blue at concentration 0.5µg/ml with compound material LP, LC, L+ Ci due to their adsorption property.

6.3.1 ANTISTAINING ACTIVITY WITH TOOTH MOULD

Table 6.4 Tooth Anstaining activity of compound material (LP,LC,L+Ci)

S.NO	Compound Material	Result
1	LP	Moderate
2	LC	Moderate
3	L+ Ci	Moderate

Tooth anti- staining of compound material 1,2 and 3 gives a moderate result due to usage of water solvent instead of alcohol.

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