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Oral tooth anti staing activity for oral hygiene using activated charcoal from Lemon Peel

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ABSTRACT: This research has been focused on cost-effective, efficient, and economic, environmentally friendly herbal source. To the present study Locally available lemon peel samples as wastes were chosen and converted to activated charcoal as a new natural compound for teeth whitening. Citrus limon (lemon) belongs to family rutaceae and is well acquainted nutritive and therapeutic property widely found in Asian countries, and their byproducts which ought to have a primary source of various bioactive compounds such as like essential oils , water soluble pectin, and insoluble antioxidants .These activated charcoal of lemon peel along cinnamon (ALC) has gained our interest in learning the potency of the teeth whitening behavior and to determine their antibacterial potency against selected gram positive bacterial strains (+) Staphylococcus aureus (S. aureus)and gram negative bacterial strains Escherichia coli (E. coli), using the disk diffusion method. On further scaling up our research, the compound showed promising antioxidant activity of both activated carbon of lemon peel with cinnamon (ALC) . In this present study, MB method were selected as standard against the developed herbal preparation, ought to be chemisorption mechanism and a one of the adopted testing methods for tooth stain removal , which is cost effective, reliable and sustainable and classical bioadsorpbent technique in tooth stain removal procedures.

KEYWORDS: Lemon Peel, Activated Charcoal, antioxidant, antibacterial, , oral anti-staining activity

I. INTRODUCTION

To date, Self-care, fastidiousness has become ones elevated alertness as an essential aspect towards dental care . This new affluent has reached dental esthetics, with tooth color being most significant.

Oral health is fundamental to universal well-being and re-counts to the brilliance of life that ranges afar the purposes of the craniofacial complex. There is a significant indication

connecting deprived oral health to chronic situations, for example, there is a strong connotation among severe periodontal diseases and diabetes.

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. [1,2,3,]. There are number of techniques are offered, to modify a tooth's color, comprising veneers, artificial crowns, and teeth whitening. The first two techniques have the disadvantage of being invasive and removing portion of the dental tissue. However, tooth whitening does not reduce the hard tissues of the tooth[4]

The essential point about the activated charcoal that quandaries to all deposits on the tooth surface, retentive of plaque, bacteria, and extrinsic stains eventually brushing leaves the tooth surface with no deposits. They can accomplish a whiter appearance by removing surface stains and plaque due to their abrasive action. Nevertheless typically, these activated carbons do not change the intrinsic color of the tooth, which is closely evaluated by the dentin color. [5,6]

The Botanical medicines have been used traditionally by herbalists and indigenous healers worldwide for the prevention and treatment of several disorders. [7]

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

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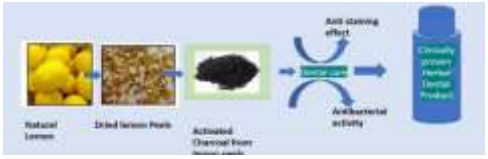
diseases, a major health issue in the world, are economically affecting people of developed countries as 10 % of the health expenditure is related to dental care.

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. [8]. Lemon is native to southeast asia ,but it is been cultivated around the world , because of its extraordinary medicinal property since ancient times and the tree is medium small and their leaves arranged in a alternative style and have a strong sweet citrus smell in leaves, flowers and fruits. Thefragnant flowers appeared to be single, or twoor more, white on top and purple on underside with 4 or 5 petals. The shape of the citrus lemon is round, spherical, citrus seeds have hard outer seed coat which are white to greenish in colour. World citrus production was almost 140 million tons and growing.[9]

Research based proven medicinal properties of lemon are to reduce high blood pressure, mental health, respiratory problems, arthritis and rheumatism, mitigation of kidney stones [10 ,11]

The peel of citrus fruits is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants.it was reported that lemon peel contains polymethoxylated flavones that are responsible for anti-inflammatory activities, and reduced capillary fragility and anti-cancer, anti-viral, [12]



Scheme :1 Schematic representation of activated charcoal Lemon peel for oral hygiene

Parallelly, The literature search reveals that no work has been done on activated charcoal composition and antimicrobial activity of nearby available left out materials of lemon peel samples. Therefore,the aim of this work is to utilize lemon peels and activated charcoal from lemon peels compounds along with cinnamon isolate and identify the chemical compounds from locally available lemon peel and determine oral anti-staining agent, antimicrobial potency against the selected bacterial strains and antioxidant activity.

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 show strong antimicrobial activity [13] which will be influenced in present study for their effective antimicrobial and anti-staining agent in oral health disorders

A new product activated Charcoal as whitening agent was small as it was particles execute on the surface of the stained tooth, the stain can be removed, this abrasive mediates to be physically firmer than the stain, leaving the tooth surface hygienic. Through this mechanism, abrasive cleaning effects only extrinsic stains and does not impact intrinsic discoloration or normal tooth color. Consequently, the capability of activated charcoal-rich toothpastes to whiten teeth derives from the elimination of extrinsic stains, but at no time by altering the intrinsic color of the dentin or enamel Therefore, the bleaching effect ascribed to activated charcoal originates from its abrasive effect. [14,15,16].

The selected studies had a moderate-high risk of bias since none of the studies performed a randomization process. Guerrero-Gironés et al., studied and proposed the biocompatibility of activated charcoal in contact with oral cells such as human gingival fibroblast could be evaluated [17]. Based on a few studies activated charcoal is listed as a new product, and more studies are needed to continue evaluating its effectiveness and safety. Other characteristics such as morphological modifications of the tooth surface produced by toothpaste materials, the influence of the toothpaste components on the whitening properties, and the durability of tooth whitening after stopping the use of the whitening toothpaste should be studied .

Scheme :2 **Applications of Lemon Peel**

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II. MATERIALS AND METHODS

Collection of Lemon Peel

Lemon peel is derived from the fruit of citrus limo. Lemon peel (citrus lemon) was utilized for the present study, Lemon peel were washed with deionized water, where then sliced and dried under the sunlight for 12hrs.The dried lemon was subjected for pulverized at moderate rotation in a regular blender into small pieces , and the powdered material to reach 200-100 µm. it was then carefully collected and stored at room temperature in an airtight bottle.

Chemicals:Acetone, Methanol, Hydrogen Peroxide, Ascorbic acid, Methylene Blue MB are purchased from Sigma Aldrich lab, Analytical grade .10mg/10mlof dye stock solution was made with redistilled water and diluted as needed to make a working solution.

Instrumentation: FTIR analysis of the samples were carried out by FTIR instrument Agilent Cary 630 FTIR , micro lab software version for the examination of the spectra. The adsorbent was characterized using Fourier Transform Infrared (FTIR) spectroscopy and identified functional groups, from Sathyabama Institute of Science and Technology.

PREPARATION OF ACTIVATED CHARCOAL

Dried Lemon Peels were collected, which is thoroughly washed with deionized water and separately dried in the oven at 110℃ for 24hrs. The dried were then pulverized into small particles sizes ranging from 200-400µm equal amounts of lemon skins were mixed and properly homogenizedbefore impregnation with an equal volume of 85% H_3PO_4. The excess liquid was removed and the material was dried for 24hrs in the oven at 110℃. Therefore, the carbon was placed in a furnace at 600℃ for 3hrs. The chemically activated carbon was cleansed using distilled water and dried at 110℃. 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.

Collection of cinnamon

Dried cinnamon bark was taken from a kitchen store. The cinnamon bark was prepared using a blender for size reduction and this powder is called general/crude powder of cinnamon. The powder colour 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 airtight bottle.

III. RESULTS AND DISCUSSION

Antibacterial study

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, Staphylococcusaureus ATCC 29213 and Klebsiella pneumoniae ATCC 700603

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)

Preparation of Bacterial inoculum

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

Antibacterial Activity

The antibacterial activity was analysed by well diffusion method. Using sterile cotton swab, the test bacterial inoculum was seeded on the Muller HinterAgar plates. The wells were made using corkborer.100 µl of samples werepipetted out in respective wells and incubate the plates at

37 °C for 24 hrs. After incubation, zone of inhibition (ZOI) around the wells were measured in diameter (mm) and noted.

The Lemon peel obtained from lemon fruit samples collected from the locally grown were used for antibacterial activity against two pathogenic bacterial strains. The presence or absence of inhibition zones based on Ciprofloxacin standard was measured quantitatively for the calculation of percentage zones of inhibition.

Our observations showed that most of the prepared concentrations of Lemon peel, activated charcoal lemon peel, activated charcoal -cinnamon did not give any zone of inhibition against pathogenic bacterial strains.

The activated charcoal lemon peel at the concentrations of 250 mcg/ml and 125 mg/ml gave very small activity against S. aureus bacteria. On the other hand, the activated charcoal lemon peel with cinnamon at the concentration of 250 mcg/ml and 125 mcg/ml gave very high zone of inhibition against S. aureus bacterial strain. However, the activated charcoal lemon peel did not give any

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activity against E.coli at any of the applied concentrations.

Interestingly the activated charcoal lemon peel with cinnamon also gave small inhibition against E. coli at the concentration of 250 mcg/ ml.

However, both the of Lemon peel, activated charcoal lemon peel, againstE.coli bacterial strain did not give any activity at all applied concentrations. This implies that the activated charcoal lemon peel with cinnamon demonstrated to have strong antibacterial potency against gram positive bacterial strains (+) Staphylococcus aureus (S. aureus) compared to gram negative bacterial strains Escherichia coli (E. coli) which is very negligible activity. All the compounds against Klebsiella pneumoniae did not give any activity at the applied concentrations. (**Table.1**)

Chemical composition and antibacterial potency

Antibacterial potencyfrom Lemon peel. Lemon peel Ac. Charcoal, Lemon peel Ac.charcoal -cinnamon **Table :1**

Compounds	concentration (mcg/ml)	*E.coli* ATCC 25922	*Staphylococcus aureus* ATCC 29213	*Klebsiella pneumon* ATCC 70
L Peel	500	nd	nd	nd
	250	nd	nd	nd
	125	nd	nd	nd
	62.5	nd	nd	nd
	31.25	nd	nd	nd
L – ac. charcoal	500	nd	nd	nd
	250	nd	6 ± 0.32	nd
	125	nd	5 ± o.15	nd
	62.5	nd	nd	nd
	31.25	nd	nd	nd
L.ac.charcoal-cinnamon	500	4 ± 0.12	16.5 ± 0.42	nd
	250	5 ± 0.28	16.5 ± 0.15	nd
	125	nd	15± 0.32	nd
	62.5	nd	nd	nd
	31.25	nd	nd	nd

nd = signifies not detectable

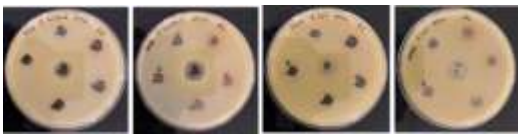

Fig 1: antibacterial activity test

ANTIOXIDANT ACTIVITY -HYDROGEN PEROXIDE METHOD

The compounds are Dried lemon peel with charcoal (LC), Dried lemon peel powder (LP) Dried lemon peel powder with cinnamon powder (L+Ci)The compounds were prepared by using various solvents methanol (70%), To the solvent (methanol) stock solution.

To the varying diluted concentration, added to 3.8ml of 0.1 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 after10min.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 =

(Absorbance in control –Absorbance in sample)

Absorbance control x 100

Phenolic compounds existing in fruits and vegetables peels are capable of neutralizing free radicals and in that way avoid the onset of degenerative diseases. Components such as tetrazene and coumarins present in lemon peel are

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capable of scavenging free radicals either by electron or hydrogen-donating mechanisms, breaking the chain reaction or removing the ROS and RNS initiator by extinguishing chain initiator catalyst.

It is evident that **Table 2** indicates that methanolic extracts showed free radical scavenging activity that increased with rise in concentration. The IC50 value of methanolic extract of lemon peel was 94.45 µg /ml, LP Charcoal 94.53 µg /ml, , LP Activated .Charcoal cinnamon 58.87 µg /ml, Unlike studies have shown that reactive oxygen species or free radicals present in the human organs root to oxidative damage to several molecules such as lipids, proteins and nucleic acids thus give way to numerous degenerative diseases.

ANTISTAINING ACTIVITY

Sampling for antistaining

According to Denga Ramutshatsha-Makhwedzha et al (2022) study ,the characterisation was developed on the compound materials(Lemon peel, activated charcoal LP and LP activated charcoal - cinnamon)for the ultimate removal of methylene blue (MB) over the testing teeth mold. Interestingly the prepared material was beneficial and effective due to its permeable composites and optimal pH conditions (2-8) to remove the adhered dirt and in vanishing the stain appeared on the teeth mold.

The activity was observed the antistaining activity of herbal composites against standard Methylene blue dye over the time scale from 0 seconds to 10 minutes.but there were remarkable achievements of antistaining activity that was observed with1- 2 minutes, and the results were unchanging after 2 minutes.

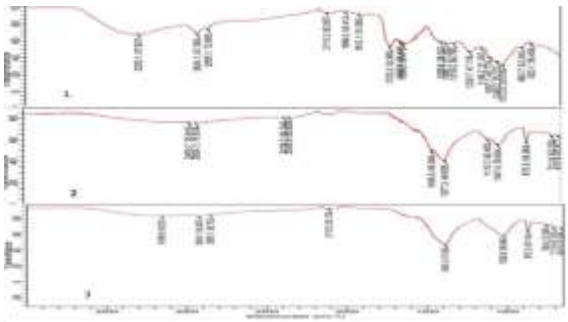

Fig 2.FTIR Spectra

1. Dried LP, 2.Dried LP activated Charcoal,
3.Dried LP activated Charcoal Cinnamon

Hence the study exhibits the promising and maximum adsorbent capacity and stain removal ability by the LP Activated charcoal cinnamon composite and the LP , and LP activated Charcoal showed minimum ability to vanish the stain on the testing teeth mold.For testing the antistaining activity , Compounds involved are Dried Lemon Peel Powder , Dried Lemon Peel Charcoal Powder, Dried Lemon Peel powder + cinnamon Powder(10µg/ml-100 µg/ml,) single teeth molds were used .Methylene blue (MB)dye (0.5 mcg/ml)

Preparation of MB Stock solution

Weighed accurately 10mg of methylene blue and diluted with 10ml of water. Shake it until it was completely dissolve. This 10mg /10ml solution was used as stock . control was prepared separately .

Preparation of Herbal composite compounds (1-3)

Weighed accurately of 10 mg of each compound material (1 ,2 and 3) was measured and diluted with 10ml of water and it was shaken well until its completely dissolved. From the stock solution, it was diluted to required concentration (10 to 100 µg/ml) of compound material was prepared.

To the methylene blue (0.5 mcg/ml), added separately the prepared Compounds (1-3) (10µg/ml-100 µg/ml) and demonstrated its adsorption activity or tooth antistaining activity against stained teeth

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molds which was subjected to mechanical brushing and the time was observed to evaluate its antistaining activity.

On the application of methylene blue dye complex with the compounds (1-3),it was observed that there were decent adsorption activity or stain removal from the stained teeth.

Henceforth the study subjected to evaluate a simple means of time dependent result for the antistaining activity.

Among all the compounds (1-3,) Dried lemon peel charcoal (LC) exhibit its antistaining activity as effective and a promising compound for stain removal in the dirt teeth compared to Dried lemon peel powder+ cinnamon powder, and dried lemon peel. (Table :3)

FTIR characterization

The spectra were scanned over the wavenumber range 4000-500cm^{-1}. for the prepared samplescomposite 1.Dried LP, 2.Dried LP activated Charcoal, 3.Dried LP activated Charcoal Cinnamon .The region of 3400-3200cm-1 indicates that symmetric and asymmetric stretching of polymeric hydroxyl group (O-H), H bonded stretching, which is characteristic of polyphenolic compounds. In the region of 2940-2925cm-1 the -CH, -CH2 and -CH3 stretching vibrations derived from carbohydrates and related compounds derived from extracts. The effect of functional groups of activated Carbon and presented in spectra (2) of Fig.2. It would be observed that the peaks at 110, 1137, 1459, 2970 cm-1 wavelengths were assigned to stretching of C-OH phenolic compounds. The peaks 1400- 900 cm-1is attributed to specific functional groups, like C-H, C-O, C-N, bonds, The lemon peel samples were seem to be complex and diverse , and the strong overlapping peaks in LP-Cinnamon Activated carbon , re due to diverse chemical complex composition and thus identifying these component is challenging. The spectra (2) showing peaks 1110,1137,1459,2970 cm-1were assigned to stretching of C-OH groups. The increase in these bands indicates the modification of activated carbon developed surface oxygen functional groups on activated carbon.

Table:2 Antioxidant activity of L.Peel ,L.Charcoal, L -Charcoal- cinnamon with methanol

S.NO	**Concentration (µg /ml)**	Inhibition of Sample Absorbance (%)		
		L Peel	L-Charcoal	L Charcoal-cinnamon
1	**50**	97.81±0.91	50±0.27	35.31±0.55
2	**100**	94.80±0.32	94.17±0.35	78.26±0.46
3	**200**	96.31±0.21	99.23±0.62	78.93±0.78
4	**300**	94.57±0.73	93.62±0.11	75.02±0.33

Ascorbic acid was used as the standard , All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM ,IC50 value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions.

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Table:3
Method of tooth antistaining by the prepared compounds (1) (2) (3) for two minutes.

Methylene Blue Concentration (µg/ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	**outcome**
Compound(1-3) (µg/ml)	10	20	30	40	50	60	70	80	90	100	
	Time taken to decolourise (sec-2 min)										
Dried lemon peel charcoal. (LC)	0.22	0.35	0.38	0.52	0.78	0.82	0.92	1.21	1.30	1.32	**Effective and best**
Dried lemon peel powder. (LP)	0.52	0.57	0.89	1.40	1.48	1.45	1.31	2.01	2.14	2.34	**good**
Dried lemon peel powder+ cinnamon powder (L+Ci)	0.99	1.13	1.24	1.26	1.35	1.47	1.53	1.81	1.96	1.99	**good**

IV. CONCLUSION

As a continuation of our study Lemon Peel, Activated Charcoal LP, Cinnamon-LP activated Charcoal were prepared for varying composites and the found that LP Activated charcoal having higher antioxidant activity, comparatively and the antibacterial activity of the activated charcoal lemon peel with cinnamon demonstrated to have strong antibacterial potency against gram positive bacterial strains (+) Staphylococcus aureus (S. aureus).our results contributed to the promising selection of LP activated charcoal Cinnamon composite showing the effective and efficient anti staining activity in removing the stains adhered to the tooth by Methylene blue adsorption technique . The present study lead the researchers and scientists to exploit the potential utilization of lemon peel activated charcoal as natural, and economic source as lemon peel comprises of flavanones and many poly-methoxylated flavones exhibited promising oral anti staining, potent antioxidant and antibacterial activity.

Declaration of competing interest

The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this paper.

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