“ANTIBACTERIAL AND ANTIFUNGAL” ACTIVITY OF THE CRUDE COCONUT SHELL OIL

SodhaRohit,GaonkarSidhesh,Dr. Kolte Satish*

(skolte2010@gmail.com,rohitsodharoxxx@gmail.com, gaonkarsidhesh99@gmail.com)

Department of Chemistry, Kishinchand Chellaram College, Churchgate, Mumbai- 400020.

Abstract:

Coconut is studied for its beneficial health effects as antitumor, anthelmintic, antimicrobial, antiseptic, antioxidant activity(1). Our main motto of this research project was to study the scientific approach to the traditional activity that is carried out in villages for treating skin infections. Our project was mainly divided into 3 parts-first the extractions of oil, second the purification of the crude extract and third, application and testing of the extracted oil.

The coconut shells were cleaned and crushed and then subjected to extraction by heating the shells in an earthen pot. The method of extraction used for the project is by referring the patented method.[2] Then Petroleum ether extract, chloroform extract, and methanol extract were obtained by solvent extraction. These extracts were studied in vitro. The ditch plate method was used to find the probable results of anti bacterial activity and the conformation was done with the agar cup diffusion method. The zone size obtained was compared with the other extracts and crude oil to compare and to explore the best antibacterial agent. Along with this the samples were tested for antifungal activity by Agar Cup diffusion method. The column chromatography was carried out for the petroleum ether extract.The attempts were made to separate the active fractions from petroleum ether exact to study the chemistry behind the activity. The eluted samples were analyzed for presence of probable active compound by thin layer chromatography (TLC). The samples which showed single spot for Thin Layer Chromatography were further monitored with the help of some chemical analysis to determined presence of nitrogen, chlorine, sulphur or any other halogen present in the sample. The samples which gave single spot for TLC were tested for the probable functional group and elements by Infrared red spectroscopy. Gas Chromatography, Mass Spectroscopy (GCMS) was used to find the probable molecular structure of separated fractions.

INTRODUCTION:

Coconut is a palm type of tree growing in on the tropical region of the countries of the world. It grows about 30m in height near the coast [(2)]. Early Spanish explorers called it coco which means "monkey face" because the three indentations (eyes) on the hairy nut resemble the head and face of a monkey. Nucifera means "nut-bearing [(3)].The tree is grown across the world cost. The wide spread of growth was due the explorers and immigrants and also due to the way of dispersal of the seed that is due to floating in the water and reaching far shore around the world [(4)].Over the years, we have used its leaves for thatching our roofs, its wood to build houses, coir for making ropes, bark as fillers in cement industry, and its fruit to quench thirst and also as plasma expanders [(5)] in medical emergencies. The nut contains white meat and sweet water.

MATERIALS AND METHODS:

Raw materials:

Coconut shells from south Mumbai were collectedrandumly. They were cleaned and sun dried. Then the cleaned shells were polished with paper to make the surface of the shells smooth. Then the shells were grinded manually to make small pieces which will be used in extraction of the crude coconut oil.

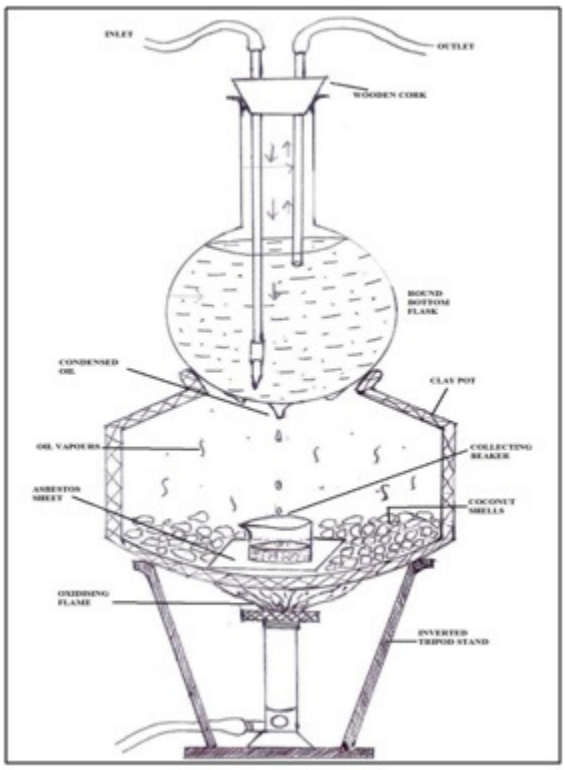
Chemicals

Analytical grade: Petroleum ether, Chloroform and Methanol were used as solvents in the extraction and chromatographic procedures. Nutrient Agar media was bought from Hi-Media, India (Mumbai). Samples for determining anti-bacterial and anti-fungal activity were prepared using Analytical grade solvents.

Bacterial culture

Two bacterial cultures, *Escherichia coli,Staphylococcus aureus and two fungal cultures, Aspergilus and Rhizopus were* procured from Microbiology Department of Kishinchand Chellaram College, Churchgate, Mumbai. They were sub-cultured using the growth medium i.e. Nutrient Agar Medium (For bacteria) &Saboraud's Agar Medium (For fungi). They were carefully preserved to prevent any kind of cross-infection and contamination.

Extraction of oil:



About 250gms of shells were heated in the earthen pot for a span of 5hrs giving a yield 25cc of oil.

Fractionation of the oil using the solvents

5gms of crude oil was taken in a separating funnel with 20ml of petroleum ether and was shaken vigorously for 5-10mins. There is a formation of distinct layer of petroleum ether which is then separated in a dish and the solvent is then evaporated. This procedure is carried out for a number of times till the batch of petroleum ether is remains colourless after shaking.

The residual oil in the separating funnel is then subjected to the extraction procedure with chloroform the same way as done for petroleum ether.

The remaining oil in the separating funnel is dissolved in methanol

All the three plates are kept for evaporation of the solvent. We obtained yellow coloured extract from petroleum ether, black coloured extract from chloroform and methanol and were named as Petroleum ether extract, Chloroform extract and Methanol extract respectively.

Chromatographic separation

A 75cm long column was taken for separation of the extracts obtained using column chromatography. 5.5gms of silica gel was taken and activated in oven at 105^0C. 5gms was soaked in the solvent, i.e. Petroleum ether and 0.5gms was mixed with the petroleum ether extract. The sample was eluted in the column by using solvent, in the sequence…petroleum,varying composition of Petroleum ether and chloroform from 90:10 to 10:90and finally with only chloroform. The eluted samples were collecting in stopper tubes and named as TT1, TT2 and so on. For further analysis of the eluted sample, they were immediately tested using thin layer chromatography and the spots were observed U.V. lamp and using iodine chamber and recorded to monitor the separation of compound.

Chemical analysis of the sample:

Organic spotting was done for the selected fractions obtained from column chromatography to detect the presence

ofelements like nitrogen, chlorine, sulphur or any other halogens.

Spectroscopic study:

For indentation of active compounds present in the sample, they are analyzed by IR Spectroscopy and Gas Chromatography Mass Spectroscopy.

Antimicrobial activity:

For checking the antimicrobial activity ditch plate method was carried out first to check whether the oil shows any positive results. And the potency was confirmed by Agar cup diffusion method.

In Ditch plate method a ditch is dug across the vertical axis of the plate. Approximately 5ml of molten agar with the insoluble sample in poured in the plate. Culture is streaked across horizontal axis. Incubation is carried out at 37^0C for 24hours. We observe for zone of clearance on and near the ditch for positive results.

In agar cup diffusion the culture is added to the molten agar and is mixed well, then added to the petri plate. After cooling, wells are bored with a cork borer. Four wells were bored for petroleum ether extract, chloroform extract, methanol extract and a control. The plates are incubated at 37^0C for 24hours.

RESULTS AND OBSERVATION:

INFRARED SPECTROSCOPY:

From the IR spectra shows peaks for the presence of Alkanes, Alkenes,Alkynesand carboxylic acid in the petroleum ether extract whilefor the selected fractions obtained from the column chromatographic separation of petroleum ether extract showed presence of more than one functional groups.Example, TT29 had alcohol/ ester group with the alkynes and alkenes; TT32 had carboxylic acid, alcohol/ester/ether with alkynes, Alkanes and alkenes; TT33 had a special peak for Monomeric alcohols with carboxylic acid, alcohol/ester/ether with alkynes and alkenes; TT32 (IV) had carboxylic acid, alcohol /ether with alkynes and alkenes.

GCMS:

GC chromatogram showed none of the fraction pure. Mass Spectroscopy of samples TT29(I), TT32(VI) and TT33 were carried out, though the pure compound could not be detected, following are the molecular weight of the compounds obtained at different time interval along with the mass spectrographs of the above mentioned samples.

TT29 (I)

Retention time	Molecular weight
1.24	119
1.31	117
1.41	119
5.85	553
6.88	604
7.74	627
8.95	678
9.43	713
9.98	764
11.43	857
11.99	878
13.86	891

TT32(VI)

Retention time	Molecular weight
1.24	119
1.31	117
1.41	119
5.85	553
6.88	604
7.74	627
8.95	678
9.43	713
9.98	764
11.43	857
11.99	878
13.86	891

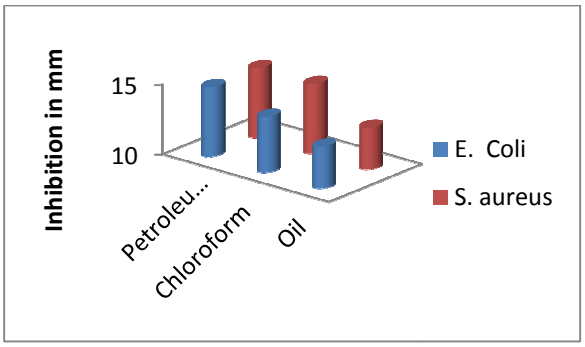
TT33

Retention time	Molecular weight
1.16	101
1.31	118
6.52	405
7.95	429
8.53	429
9.02	471
9.23	429
11.01	505
13.21	479
14.04	503
14.78	539

Anti- bacterial activity:

Ditch plate

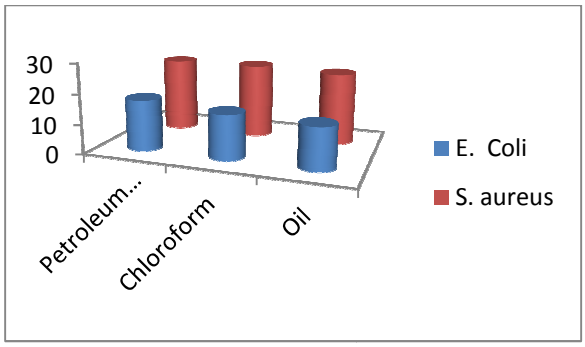
Sample	S. aureus (1+2+3)	E. coli (1+2+3)
Petroleum ether	15	15
Chloroform	13	14
Oil	12	13



From the ditch plate method indicates that petroleum ether is equally effective on both S. aureus and E. coli. The chloroform extract is less effective on E. coli than on S. aureus while the oil is more effective on E. coli than on S. aureus.

Agar cup diffusion

Sample	S. aureus (1+2+3)	E. coli (1+2+3)
Petroleum ether	25	17
Chloroform	25	15
oil	24	14



The agar cup diffusion method indicates that Petroleum ether is more effective on S. aureus than on E. coli chloroform is more effective on S. aureus than on E. coli. Oil sample has also shown a better inhibition on s. aureus than on E.coli.

Anti-fungal activity:

The sample show positive results for both the fungi used Rhizopus and Aspergillus

CONCLUSIONS:

The samples were tested for their anti bacterial and antifungal activity. From the experimental resultsit can be concluded that the petroleum ether extract is effective on S. aureus which is responsible for the skin infections. It also gave satisfactory result for E. coli which is a part of normal intestinal flora. Furtherer testing needs to be done to know the molecules responsible for this activity. Comparative study needs to be done for the antifungal activity too.

Future Prospectus:

Since the active compound was proved to be impure from IR and GCMS, it boosts the researcher to bring about the effective chromatographic separation, to could to find the most active component.

Formulations can be made using the oil extracts to cure skin infections caused by *S.aureus,* which was the motto of our project. Other than that, since the extracts gave satisfying results for the other bacteria i.e. *E.Coli*& Fungus like *Aspergillus* & *Rhizopus,* formulations can be made to use these extracts for infection by these micro-organisms.

REFERECNCE

1. Antioxidant & Antimicrobial Activities of CocosNucifera Linn. (Arecaceae) Endocarp Extracts, Rajeev K Singla, Nitesh Jaiswal2, VaradarajBhat, Hitesh Jagani3, Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal-576104, Karnataka, India, Department of Medical Microbiology, Kasturba Medical College, Manipal University, Manipal-576104, Karnataka, India, Department of Pharmaceutical Biotechnology, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal-576104, Karnataka,India
2. http://www.ehow.com/list_7210158_uses-coconut-shells.htcm3
3. http://www.blueplanetbiomes.org/coconut.htm
4. http://faculty.ucc.edu/biology-ombrello/POW/coconut_palm.htcm3
5. Influence of coconut water on hemostasis.Am J Emerg Med. 2001 Jul;19(4):287-9.