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Antibacterial and Antifungal Activity of Crude Coconut Shell Oil

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Abstract

Traditionally coconut shell oil has been used for the treatment of skin infections. It has been used for antitumor, antihelminthic, antimicrobial, anti-inflammatory antiseptic and antioxidant activity. The aim of the project was to extract oil from the coconut shell, purify it and test it for anti-bacterial and anti-fungal activity. The shell oil was first extracted by modified method then it was subjected to sequential solvent extraction with petroleum ether, chloroform and methanol. The anti-bacterial and anti-fungal activity was checked by ditch plate and agar cup diffusion method. The microbial activity was found to more active in Petroleum Ether extract, hence an attempt was made to separate the components present in it by Flash Column Chromatography. The monitoring was done with TLC, and pure components were further studied for identification of Functional groups present in it using FTIR.

Keywords: Cocus nucifera, solvent extraction, flash column chromatography, tlc, ftir.

Introduction

Coconut (Coccus nucifera) is a palm tree growing in the tropics. 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. The nut contains white meat and sweet water. These have anti-bacterial and antifungal, anti-viral anti-inflammatory[1,2], *antioxidant*[3] activity. Antimicrobial activity of the water extract of coconut husk has already been demonstrated[4]. However, studies regarding the polyphenol content of the coconut fruit wall are limited[5]. Coconut shell can be grinded into powder and used as a filter for synthetic resins. It is also used as activated carbon and charcoal. Oil derived from coconut shells have been shown to have antimicrobial and anti-fungal activity[6]. Oil extracted from the darnel in concentration of 5% to 40% (w/w) inhibited bacterial activity against E. coli, *Bacillus subtilis*[7,8.]

Material and Methods

Raw materials: Coconut shells from south Mumbai were collected. They were cleaned and sun dried. Then the cleaned shells were polished with paper to make the surface of the shells smooth. Further the shells were ground manually to make small pieces which were 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 procured from Hi-Media, India (Mumbai)

Bacterial cultures: Two bacterial cultures, *Escherichia coli, Staphylococcus aureus and two fungal cultures, Aspergillus niger and Rhizopus stolonifer* 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) and Sabourauds Agar Medium (For fungi). They were maintained at 4^0C.

Extraction of oil: About 250gms of ground shells were heated in the earthen pot for a span of 3hrs giving a yield of 25cc of oil shown as in figure-1[9, 10].

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 5-10 minutes. Yellow colour development in the petroleum ether indicates extraction 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 till colourless chloroform is obtained.

The remaining oil in the separating funnel is dissolved in methanol (since it is seen that the crude oil in completely miscible in methanol).

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

Chromatographic separation: A 0.74m long silica column was for separation of the extracts obtained by solvent extraction. 5.5gms of silica gel was taken and activated in hot air oven.

5gms activated silica was soaked in the solvent, i.e. Petroleum ether and 0.5gms was mixed with the petroleum ether extract. The sample was run in the column by using solvent, 90:10 Petroleum ether: Chloroform initially and then with decreasing concentration of petroleum ether and increasing concentration of chloroform and finally with chloroform.

The eluted samples were collected in stopper tubes and named as TT1, TT2 and so on. The elution was simultaneously monitored by thin layer chromatography and the spots were observed and recorded. TT 29 showed single spot under UV rays and I_2 vapour chamber. TT32 was further purified by flash column chromatographic method since several spots were observed. Once the solvent is evaporated yellowish orange emulsion is obtained.

Chemical analysis of the sample: Organic spotting was done for samples obtained from column chromatography to detect the presence of elements like nitrogen, chlorine, sulphur or any other halogens.

Spectrometric analysis: The samples were further subjected to IR Spectroscopy and Gas Chromatography Mass Spectroscopy which has indicated the probable function group and molecular weight.

Antimicrobial activity: For checking the antimicrobial activity ditch plate method was carried out (since ditch plate method is applicable for water soluble and insoluble compounds)

In Ditch plate method a ditch 1cm X 9cm is cut from a nutrient agar plate and it is then filled with 4ml molten nutrient agar mixed with 0.5 ml of sample oil. Culture suspensions (10^8cfu/ml) of the two bacterial cultures i.e. *E. coli* and *S. aureus* were streaked across the ditch.

The plates were incubated 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 suspension is added to molten nutrient agar and is mixed well, then poured into sterile petri plate. After cooling, wells (10mm) are made and 50µl of petroleum ether extract, chloroform extract, methanol extract of the oil were added with appropriate controls. The plates are incubated at 37^0C for 24hours.

Table-1
Microbial activity by Ditch plate methtod

Sample	**S. aureus**				**E. coli**			
	1	**2**	**3**	**Average (In mm)**	**1**	**2**	**3**	**Average (In mm)**
Petroleum ether extract	15	15	16	15	13	16	16	15
Chloroform extract	13	16	16	15	15	14	14	14
Oil	14	13	13	13	14	12	00	13

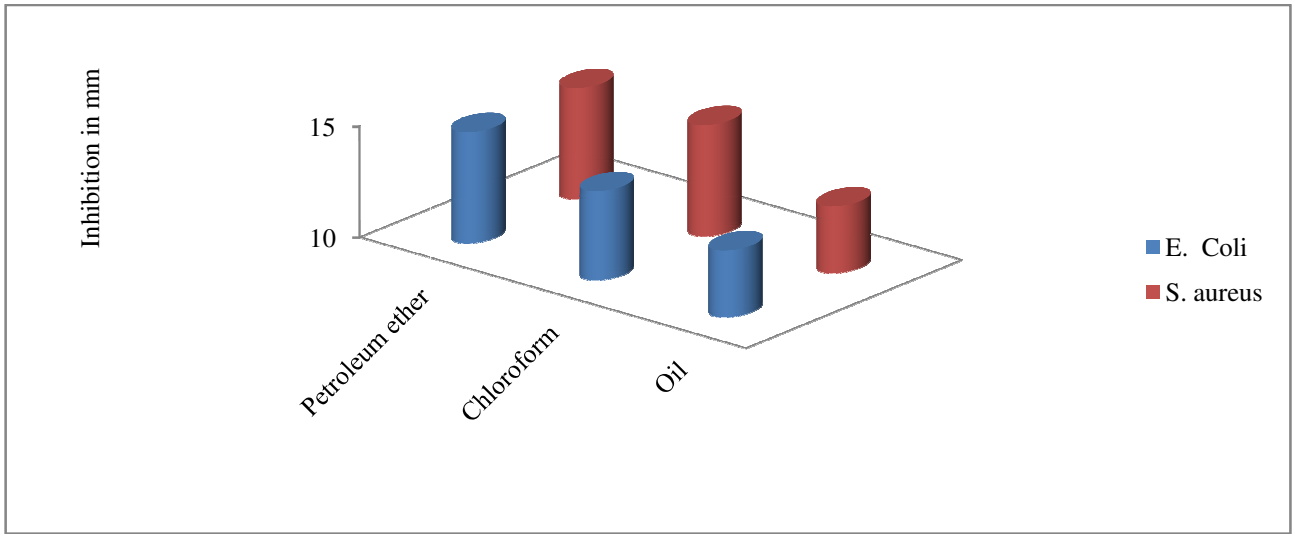

Figure-1
A graph representing antimicrobial activity of the extracts using Ditch Plate method

Table-2
Microbial activity by Agar cup diffusion method

Sample	S. aureus				E. coli			
	1	**2**	**3**	**Average (In mm)**	**1**	**2**	**3**	**Average (In mm)**
Petroleum ether extract	25	26	23	25	00	15	18	17
Chloroform extract	26	24	24	25	00	14	16	15
Oil	23	24	23	24	00	12	15	14

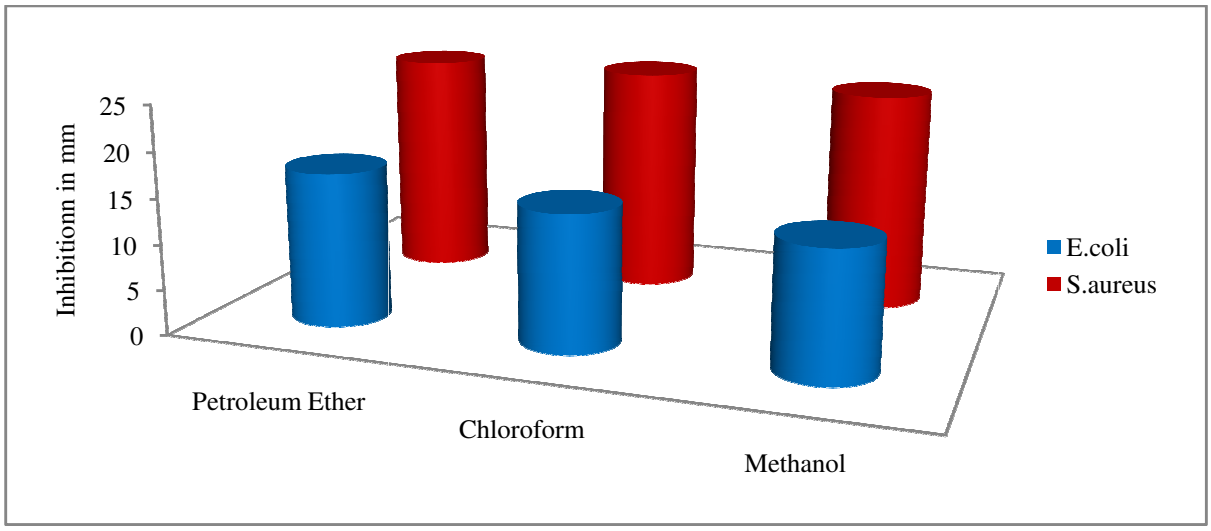

Figure-2
A graph representing antimicrobial activity of the extracts using Agar Cup Diffusion method

Results and Discussion

Infrared spectroscopy: The IR study indicates presence of Alkynes, Alkanes, Alkenes and Acid group for the petroleum ether extract. The details are as follows (figure-2, 3 and 4)

GCMS: The samples were then subjected to Gas chromatography mass spectroscopy and it was found that the samples are a mixture of molecules having close structural resemblance and molecular weight.

Anti- bacterial activity: From the ditch plate method it is observed that petroleum ether extract is equally effective on both *S. aureus* and *E. coli*. The chloroform extract is less effective on *E. coli* while the oil is equally effective on *E. coli* and *S. aureus*. From the agar cup diffusion method we come to know that Petroleum ether is more effective on *S. aureus* than on *E. coli*. Chloroform extract is more effective on *S. aureus* than on *E. coli*. Oil sample has also shown better inhibition of *S. aureus* than on *E. coli*.

Anti-fungal activity: The sample show positive results for both the fungi used *Aspergillus Niger and Rhizopus stolonifer*

Table-3
Infrared spectroscopy

Sample	Functional groups
Pet ether	Alkanes, alkenes, OH,-O-, -OO-, -COOH
TT29	Alkynes, alkenes, Alcohol ethers
TT32	Alkanes, alkenes, alkynes, -COOH, -OH, -OR, -OOR
TT32(VI)	Alkanes, alkenes, alkynes, -COOH, -OH, -O-, -COO-
TT33	Alkynes, alkenes, -OH, -O-, -COOH, -COO-, Monomeric alcohols

Conclusion

The samples were tested for their anti-bacterial and antifungal activity. Petroleum ether extract is effective on S. aureus which is responsible for skin infections. It also gave satisfactory result for E. coli which is a part of normal intestinal flora though the shell oil gave comparatively lesser inhibition. Hence we can say that the anti-bacterial and anti-fungal activity is shown

collectively by several components of the fractionated coconut shell extract. We can use the petroleum ether extract of the crude coconut shell extract as an alternative Further tests need to be done to separate and purify the compound that is responsible for the inhibitory effect.

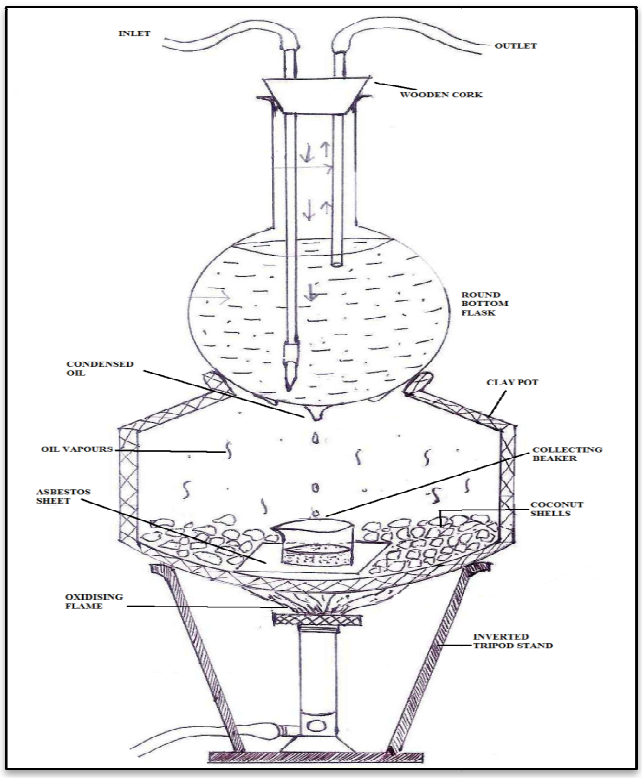

Figure-3
Apparatus for extraction of oil (IR Reports)

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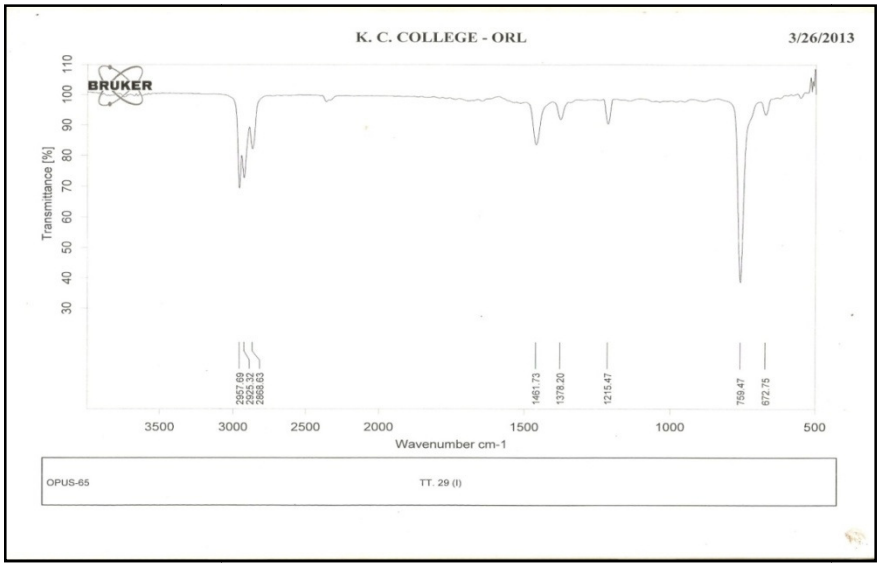

Figure-4
FTIR graph for sample TT29

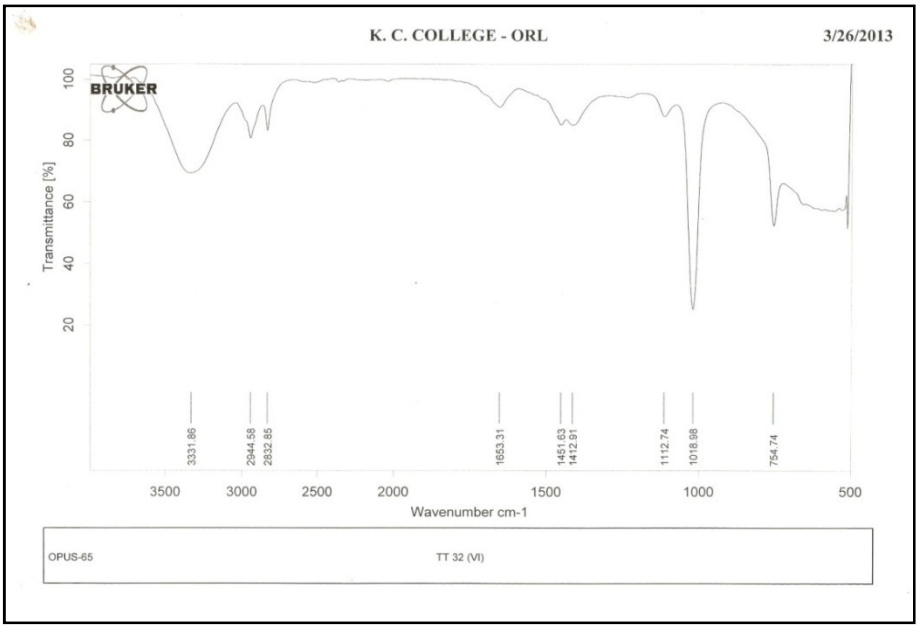

Figure-5
FTIR graph for sample TT32 (VI)

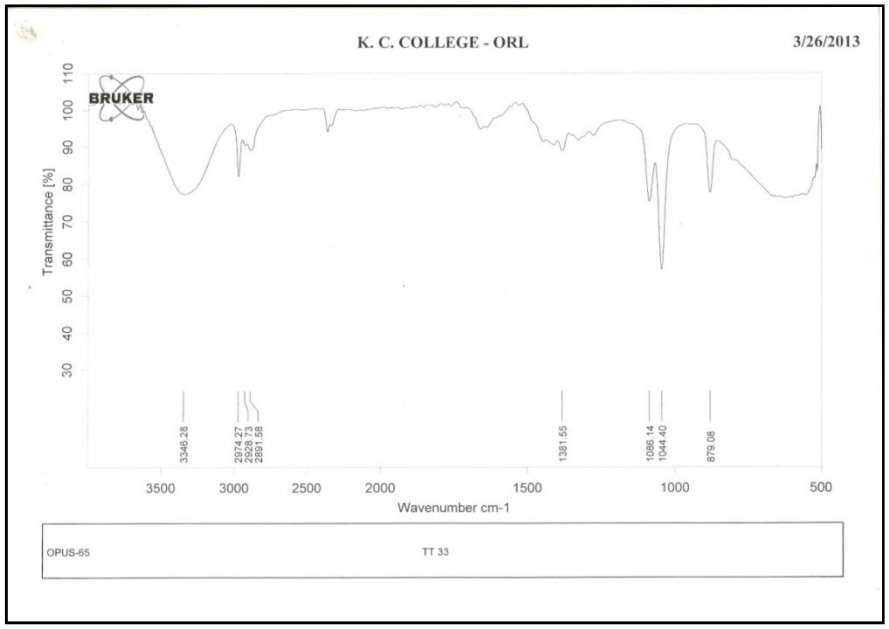

Figure-6
FTIR graph for sample TT33

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