

Formulation and Evaluation of Topical Preparations Containing Pyrolytic Oil Obtained from Local Biomass

Jithu Jerin James^{1,*}, Sandhya Kadamboor Veethil², Joysa Ruby Joseph Rajarathinam¹

¹Department of Pharmaceutical Analysis and Pharmaceutics, Acharya and BM Reddy College of Pharmacy, Bengaluru, Karnataka, INDIA.

²Department of Pharmaceutics, Faculty of Pharmacy, MS Ramaiah University of Applied Sciences, Bengaluru, Karnataka, INDIA.

ABSTRACT

Objectives: Pyrolytic oil of coconut shell was obtained by the fast pyrolysis method. This research aimed to identify the use(s) of pyrolytic oil in topical pharmaceutical formulations. The study included extraction of bio-oil by pyrolysis, physicochemical characterization, screening the oil for microbial activity, preformulation studies, formulation into suitable topical preparations like ointment, gel and cream, evaluation studies of the formulation and stability studies. **Materials and Methods:** The extracted pyrolytic oil was subjected to diversified physicochemical evaluation tests like specific gravity, acid/saponification/ester values, viscosity, moisture content, pH, thin layer chromatography, Fourier-transform infrared spectroscopy and Gas chromatography-Mass spectrometry analysis. It was then screened for its potential for antibacterial/antifungal activity. Various topical formulations were prepared using the pyrolytic oil. Preformulation studies of the pyrolytic oil were carried out. This was formulated into suitable topical preparations like ointment, gel and cream and subjected to physicochemical evaluations which include appearance, pH, spread ability, viscosity and antimicrobial activity. Also, the formulations were evaluated for its stability. **Results:** The stability studies results showed no change in pH, spreadability and viscosity, thus indicating good stability of the formulations. The prepared formulations displayed good antibacterial and antifungal activity. **Conclusion:** The use(s) of pyrolytic oil have not been explored to its full potential for its pharmaceutical application. In this study, the pyrolytic oil showed good antimicrobial activity due to the presence of polyphenols. Hence it could be used as an effective topical formulation to cure various topical infections.

Key words: Biomass, Pyrolytic oil, Characterization, Antimicrobial activity, Topical preparations, Evaluation.

INTRODUCTION

Various research and development activities are being performed in the field of science to identify a substitute for fossil fuels due to increased environmental concerns associated with it. Hence, we need to find out an alternative to fossil fuels. One of the alternate sources for fuels is the use of biomasses to produce liquid fuels by fast pyrolysis. Biomass, an organic matter obtained from plants or animals is a renewable and sustainable source of energy. Its popularity is due to its local availability and cost-effectiveness. Biomass can be converted to higher-value products or energy. Pyrolysis is an irreversible

thermochemical treatment in which biomass is exposed to elevated temperatures in an inert atmosphere. The products of pyrolysis include bio-oil, gas and carbon black.¹ Pyrolytic oil contains a mixture of 300 or more compounds resulting from the depolymerisation of cellulose, hemicellulose and lignin. Pyrolytic oils are generally acidic, contain solid char particles with relatively high moisture content, low heating value, thermally unstable and degrade with time. Many value-added products, liquid fuels and chemicals can be obtained by the refinement of bio-oils.

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Correspondence:

Ms. Jithu Jerin James

Department of Pharmaceutical

Analysis, Acharya and BM

Reddy College of Pharmacy,

Soldevanahalli,

Bengaluru-560107,

Karnataka, INDIA.

E-mail: jithujerinjms25@

gmail.com



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The raw material used for rapid pyrolysis was powdered coconut shell. The coconut tree (*Cocos nucifera* Linn.) comes under Arecaceae botanical family, mostly found in the tropical zone and the fruit shell contains cellulose, hemicellulose and lignin.² Pyrolytic oil can be obtained from shells by fast pyrolysis process and the raw materials are available in abundance. The use(s) of pyrolytic oils from various biomasses is minimal and have not been used effectively in the pharmaceutical industry.

We intend to screen the oil for any microbial activity and formulate it into various topical formulations which include ointments, gels and creams for effective topical delivery. It is convenient in terms of portability to be used in treating a wide array of disorders. The pyrolytic oil obtained was purified, screened for microbiological activity and successfully formulated into effective topical semisolid dosage forms like ointments, gels and creams. Thus, in the present study, we have attempted to scrutinize and explore the uses of bio-oil for topical application. Such studies would promote the use of the locally available biomass to be used in the pharmaceutical industry either as a medicament or as an excipient.

MATERIALS AND METHODS

Chemicals

Carbopol 940 was purchased from S. D. Fine Chemicals, Bengaluru. Triethanolamine, propylene glycol, emulsifying wax, white soft paraffin used in this investigation were procured from Karnataka Fine Chemicals, Bengaluru. Analytical grade chemicals and distilled water was used for the research work.

Bacterial cultures

Microorganisms used for screening; bacterial cultures - *Escherichia coli*, *Bacillus subtilis* and fungal culture - *Candida albicans*. The microorganisms were sub-cultured using the Muller Hinton Agar (MHA) growth medium and incubated at 37°C overnight. It was then used for screening of antimicrobial activity.³

Biomass and Pyrolytic Oil Extraction

The ripe coconut shell fruits used in this study were procured from south Karnataka, India. The shells were cleaned and dried in the sun thoroughly to remove moisture before using them for pyrolysis. The shells were broken into small pieces with a rotary grinder and sieved. These were further crushed and dried in an oven for 12hr at 110°C to free them from moisture before pyrolysis. The powdered coconut shells were subjected to incomplete combustion with 30% air supply and a temperature of 450–500°C in a kiln which

was heated externally by an electric furnace to obtain producer gases and pyrolytic oil. A stainless-steel pipe maintained at 200°C (to avoid condensation) was used to connect the kiln and glass condenser (maintained at 25°C by circulating water), to collect condensable liquid products (pyrolytic oil + water). The condensed liquid obtained was then collected into a sample container, and calculated weight difference between empty container and container with liquid to get liquid weight.^{4,6}

Characterization

The characterization of the pyrolytic oil was carried out by performing various tests which included specific gravity, acid value, SAP (saponification) value, ester value, viscosity, water content, pH, TLC (thin layer chromatography), FTIR (Fourier-transform infrared spectroscopy) and GCMS (Gas Chromatography Mass Spectrometry) analysis.^{7,8} The specific gravity of pyrolytic oil was determined. Acid value, saponification and ester values were calculated via Albert's method. The viscosity was determined for 250ml of pyrolytic oil at 22.7°C using Brookfield DV-II + Pro EXTRA viscometer with T-bar spindle 91 at 50 rpm, reading was noted in centipoises (cP). The moisture content was determined by the Karl Fischer Titration method. Each experiment was performed in triplicate. The pH of pyrolytic oil was recorded. The IR fingerprint of pyrolytic oil was carried out in the range of 400–4000 cm⁻¹ with the help of Bruker Tensor 27 FTIR using OPUS 5.5 software.⁹ GC-MS was performed using an Agilent 7890B GC system equipped with an ionization potential of 70 eV, to identify the composition of the oil. For analysis, 99.99% helium (M.P) and HP-5 capillary column (dimensions: 30 m × 0.25 mm i.d; 0.25µm thickness) was used. The flow rate was adjusted to 1ml/min. The temperature of the column was programmed to reach 70°C from 25°C and maintained at this temperature for 2 min. It was then heated to 300°C and maintained temperature for 30 min. The heating rate while programming was 10°C/ min. Pyrolytic oil (2µl) was injected in split less mode maintained at 250°C. The MS scan range was 35-600 atomic mass units. NIST library was used to identify the mass of the compounds from the chromatograms.¹⁰ The bio-oil was then screened for its potential for antibacterial/antifungal activity against *E. coli*, *Bacillus subtilis* and *Candida albicans* by the agar diffusion method.^{11,12}

Compatibility Studies

The IR spectra of formulations were performed within the range of 400–4000 cm⁻¹ with the help of Bruker Tensor 27 FTIR using OPUS 5.5 software. Pyrolytic

oil, prepared formulations and excipients were placed in the sample port of FTIR separately; the spectra's were recorded. Compatibility studies were carried out to know the possible interactions between pyrolytic oil and excipients used in the formulation.

Formulation of Topical Preparations of Bio-oil

The pyrolytic oil obtained was formulated into semisolid dosage forms like gel, ointment and cream to study the relative stability of the bio-oil in various topical formulations and improve its aesthetic appeal. The prepared formulations were subjected to various evaluation studies.^{13,14}

Preparation of Gels

The gel (1.5%) was prepared by using carbopol 940 and triethanolamine. 1ml (0.99g/ml) of bio-oil was dissolved in propylene glycol (permeation enhancer), stirring continued until a uniform gel was obtained.

Preparation of Ointments

The ointment was prepared using emulsifying wax and white soft paraffin, composition of ointment is shown in (Table 1). Pyrolytic oil was added to the molten base, stirred well until it attained room temperature. The homogenous ointment obtained was transferred into a suitable container.

Preparation of Cream

The cream was prepared using emulsifying wax, pyrolytic oil and distilled water, the composition of cream is shown in (Table 2). The homogeneous cream obtained was transferred into a suitable container.

Evaluation of Topical Formulations

Physical appearance

The homogeneity and aesthetic characteristics of the prepared gel, ointment and cream were recorded.

Table 1: Formulation of ointment.

Sl.No.	Ingredients	Composition (%)
1	Emulsifying wax	30
2	White soft paraffin	50
3	Pyrolytic oil	20

Table 2: Formulation of cream.

Sl.No.	Ingredients	Composition (%)
1	Emulsifying wax	20
2	Pyrolytic oil	20
3	Preservatives	0.5
4	Distilled water	59.5

Spread Ability

The horizontal glass plate method was used to determine the spread ability of all formulations. Accurately weighed sample (2.5gm) was sandwiched between two glass plates and (5gm) standard weight was attached to the upper glass plate. The sandwiched sets were held in the vertical position. The time taken for the plate to separate from the fixed slide was noted. The formula used to calculate spread ability is mentioned below.

$$\text{Spread ability} = \frac{(\text{Weight attached to upper side} \times \text{Length})}{\text{Time taken to separate from the fixed slide}}$$

Weight attached to upper slide = 5gm; length = 8cm

pH Determination

The pH was detected using Digisun electronics digital pH meter, 7007 model, (accuracy ± 0.01). For pH determination, a 10% sample in de-ionized water was prepared.

Viscosity Measurement

The viscosity was determined using Brookfield DV-II+Pro EXTRA viscometer. Spindle LV-4 (64) fixed to viscometer was immersed into 250 gm of the sample. The viscometer was operated from 5 to 50 rpm and reading was noted in cP.

FTIR

The IR spectra of formulations were performed within the range of 400–4000 cm^{-1} with the help of Bruker Tensor 27 FT-IR using OPUS 5.5 software. Prepared formulations were placed in the sample port of FTIR; the spectra of formulations were recorded.

Antimicrobial Activity

Antibacterial activity

The antibacterial activity of the formulations was determined by the spread plate technique using the medium Muller Hinton Agar (MHA). The diameter of the wells bored was 6mm which was loaded with pyrolytic oil, positive control (Streptomycin) and formulations to different wells. *E. coli*; Gram (-) and *Bacillus subtilis*; Gram (+) bacteria were used for screening. Incubation (24hr at 37°C) was done and recorded the zones of inhibition.¹⁵

Antifungal activity

The antifungal activity of the formulations was determined by the spread plate technique using the medium Muller Hinton Agar (MHA) with 2% dextrose. The diameter of the wells bored was 6mm which was loaded with pyrolytic oil and formulations. Ciprofloxacin (5 μg) was used as a positive control for *Candida albicans*. Incubation (24hr at 37°C) was done and the zones of inhibition were recorded.^{16,17}

Accelerated stability studies

ICH guidelines were followed to carry out stability studies. For accelerated stability testing, the prepared formulations were subjected to $40 \pm 2^\circ\text{C}/75 \pm 5\%$ temperature and relative humidity for three months respectively (Stability Chamber, Thermolab Scientific Equipment's, Model No. 325A501). At regular time intervals, the samples were withdrawn regularly for three months duration. The samples were checked for a change in pH, spread ability, viscosity and FTIR. The changes observed were recorded. Each experiment was performed in triplicate.

RESULTS AND DISCUSSION

Characteristics of Coconut Shell Pyrolytic Oil

Bio-oil, a dark brown liquid with a yield of 23% was homogeneous and no phase separation was observed upon storage. The physical properties of extracted pyrolytic oil are recorded in (Table 3). The pyrolytic oil was found to be acidic and the high moisture content reduced the viscosity significantly. Pyrolytic oil contains many compounds and the initial stage identification of the compounds was made based on TLC using mobile phase: n-hexane, benzene, ethyl acetate in the ratio 20:10:1. The TLC of pyrolytic oil before derivatization (A-at 254nm and B-at 366nm) and after derivatization (C-at 366nm and D-visible light) is shown in (Figure 1).

FTIR of Pyrolytic Oil

The FTIR of pyrolytic oil is shown in (Figure 2). The FTIR analysis of pyrolytic oil given in (Table 4) describes the various functional groups, vibrations and intensity

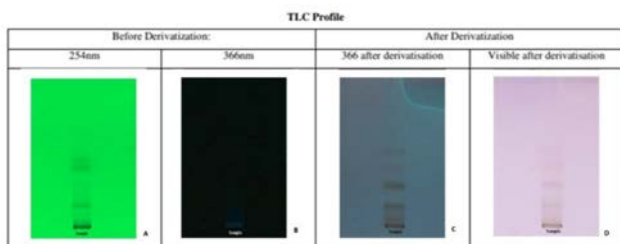


Figure 1: TLC of pyrolytic oil (mobile phase: n-hexane, benzene, ethyl acetate in the ratio 20:10:1); before derivatization (A-at 254nm and B- at 366nm) and after derivatization (C- at 366nm and D-visible light).

for the characteristic wavenumber of the pyrolytic oil. The FTIR spectrum clearly shows the presence of saturated and unsaturated hydrocarbons in the oil.

GC-MS Analysis for Composition Study

The coconut shell consists of lignocellulosic components such as hemicellulose, cellulose and lignin. During thermal degradation processes, these components are converted into different types of hydrocarbons, carboxylic acids, acid derivatives and phenols. The GC-MS chromatogram of pyrolytic oil (Figure 3) was analyzed to know the exact composition of the oil. GC-MS analysis of coconut shell pyrolytic oil revealed thirteen compounds which are summarized in (Table 5) of which phenol and phenol derivatives are the major constituents.

Compatibility Studies (FTIR)

The compatibility between the drug and polymer was compared by FTIR spectra. The position of the peak in FTIR spectra of pyrolytic oil is compared with those in FTIR spectra of pyrolytic oil with polymers and excipients as shown in (Table 4). It was observed that there was no major shift in the peaks of spectra of pyrolytic oil and polymers, which proved that bio-oil and excipients were compatible. In conclusion, pyrolytic oil can be employed with the selected polymer without causing instability in the formulation.

Characterization of Formulations

All pyrolytic oil formulations were subjected to various characterizations which included pH, homogeneity,

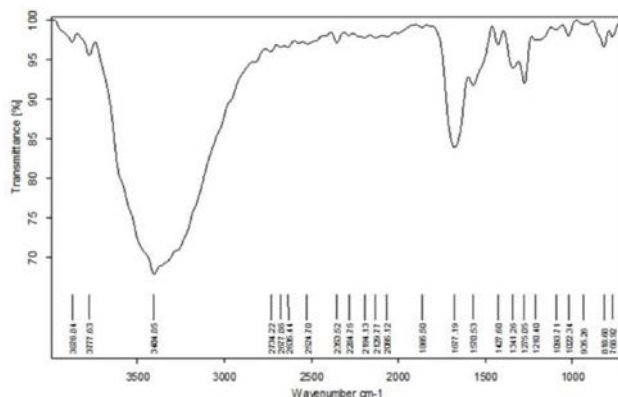


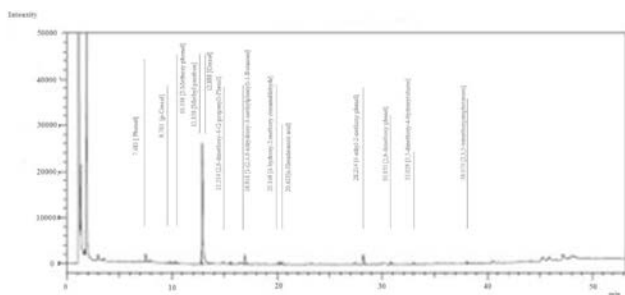
Figure 2: FTIR spectrum of pyrolytic oil.

Table 3: Physical properties of coconut shell pyrolytic oil.

Description	Specific gravity (g/ml)	Acid value	Saponification value	Ester value	Moisture content (% w/w)	pH	Viscosity (cP)
Dark brown thin liquid	0.99	140.25	332.39	192.14	70.86	2.80	12.7

Table 4: FTIR analysis of pyrolytic oil with polymers.

Functional groups	Types of vibration	Characteristic vibrations (cm ⁻¹)		Intensity
		Pyrolytic oil	Pyrolytic oil + polymers (gel, ointment, cream)	
Alcohols/ Phenols (O-H)	H bonded O-H Stretch	3400	3365-3434	Strong broad band
Alkane (C-H)	Stretch	2853 - 2962	2853 - 2962	Strong
Alkane (C-H)	Bending	1340 – 1450	1340 – 1450	Medium
Ketones/ Aldehydes (C=O)	Stretch	1650-1750	1650-1750	Strong
Alkene (C=C)	Stretch	1600-1680	1600-1680	Medium
Aromatic (C=C)	Stretch	1475-1600	1475-1600	Medium
Aromatic ether (C-O)	Stretch	1000-1300	1000-1300	Strong
Aromatic	Bend	690-900	690-900	

**Figure 3: GC-MS of coconut shell pyrolytic oil.****Table 5: GC-MS analysis of coconut shell pyrolytic oil.**

Retention time	Compound name	Area	Area %
7.483	Phenol	12429	5.3904
9.793	p-cresol	2685	1.1644
10.336	2-methoxy phenol	2307	1.0004
12.838	Methyl paraben	4161	1.8045
12.888	Cresol	165805	71.9081
15.534	2,6-dimethoxy-4-(2-propenyl)-phenol	3687	1.5991
16.918	1-(2,4,6-trihydroxy-3-methylphenyl)-1-butanone	11981	5.1959
20.146	4-hydroxy-2-methoxy cinnamaldehyde	4067	1.7639
20.433	n-hexadecanoic acid	4049	1.7562
28.214	4-ethyl-2-methoxy phenol	12914	5.6007
30.850	2,6-dimethoxy phenol	2895	1.2554
33.029	3,5-dimethoxy-4-hydroxytoluene	1655	0.7179
38.070	2,3,5-trimethoxyamphetamine	1944	0.8430
Total		230579	100.000

spread ability and viscosity. The homogeneity and pH of formulations are given in (Table 6). The spread ability of ointment and gel are shown in (Table 7). FTIR analysis of formulations was also carried out and the

Table 6: Homogeneity and pH of formulations.

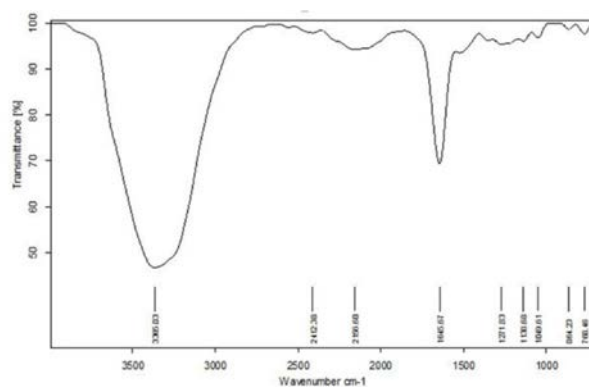
SI.No	Formulation	Homogeneity	pH
1	Ointment	Homogenous	6.3
2	Gel	Homogenous	6.6
3	Cream	Homogenous	6.8

*n=2

Table 7: Spreadability of formulations.

SI.No	Formulation	Spreadability coefficient at 30 sec
1	Ointment	1.333
2	Gel	1.667

*n=3

**Figure 4: FTIR spectrum of gel.**

FTIR spectrum of gel, ointment and cream are shown in (Figure 4 - 6). The pH of developed formulations ranged from 6.0 ± 0.1 to 6.8 ± 0.2 and was found to be near to skin pH, while viscosity values of all formulations at 23.6°C ranged from 15000 to 1800 cP. The results indicate their pseudo plastic behavior

wherein the viscosity decreases upon increasing shear rate (1.04/s to 10.45/s), thus attesting their shear thinning property. The effect of shear rate on the viscosity of formulations is shown in (Figure 7). The spread ability of ointment and gel was found to be 1.333 and 1.667 g/cm/s respectively. A shorter interval of time indicates better spreadability.

Anti-microbial Activity of Pyrolytic Oil and its Formulations

It was observed that in the agar diffusion method, the pyrolytic oil and its topical preparations exhibited inhibitory activity against the tested bacterial strains - *E. coli* Gram (-) and *Bacillus subtilis* Gram (+) and fungal

strain *Candida albicans*. Pyrolytic oil and its formulations produced a prominent zone of inhibition against the strains. The anti-microbial activity of pyrolytic oil and its formulations is shown in (Table 8).

Stability Study results of Formulations

The stability studies were done on pyrolytic oil loaded formulations and at the end of three months, samples were evaluated for spread ability, pH and viscosity. No separation of oil was seen in ointments or gels and creams. Results confirmed no significant difference between the initial and final stability study results, thus indicating the stability of prepared formulations with a shelf life of three years. The stability study results of formulations were tabulated in (Table 9).

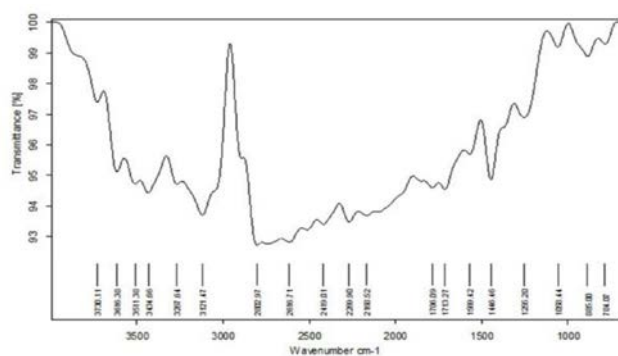


Figure 5: FTIR spectrum of ointment.

Figure 5: FTIR spectrum of ointment.

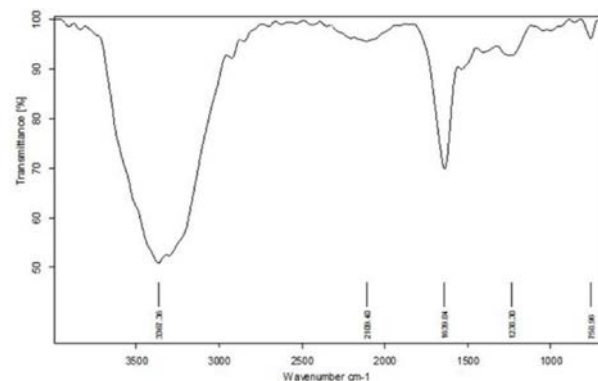


Figure 6: FTIR spectrum of cream.

CONCLUSION

Thermal pyrolysis of coconut shell was carried out and a dark brown liquid was obtained with a yield of 23.27%. The pyrolytic oil was characterized based on specific gravity, acid/saponification (SAP)/ester values, viscosity, water content, pH and found to be acidic. The values obtained correlate with the other pyrolytic oil characteristics mentioned in the investigations of niger seed and walnut shell by Shadangi and Mohanty⁷ and Zhai *et al.*⁸ FTIR analysis revealed the presence of saturated and unsaturated hydrocarbons in the oil which was supported by GC-MS analysis.

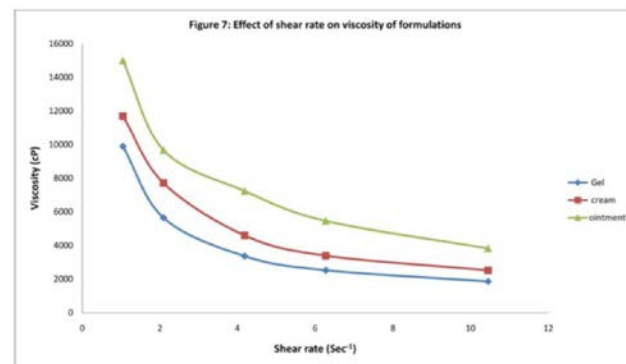


Figure 7: Effect of shear rate on the viscosity of formulations.

Table 8: Antimicrobial activity of pyrolytic oil and its topical formulations.

Test Organism	Zone of Inhibition (mm)				
	Streptomycin (10mcg)	Pyrolytic oil (10mcg)	Gel	Ointment	Cream
<i>E. coli</i>	18-19	28-30	10-11	8-10	10-11
<i>Bacillus subtilis</i>	18-19	28-30	11-12	8-10	11-12
Test Organism	Zone of Inhibition (mm)				
	Ciprofloxacin (5 mcg)	Pyrolytic oil (5 mcg)	Gel	Ointment	Cream
<i>Candida albicans</i>	38-40	38-40	14-15	11-13	14-15

Table 9: Stability study results of formulations.

Days	Spreadability (g.cm/sec) at 30 sec		Viscosity(cP) T-bar spindle 96 at 50rpm			pH		
	Ointment	Gel	Ointment	Gel	Cream	Ointment	Gel	Cream
0	1.333	1.667	2793	284	2464	6.3	6.6	6.8
15	1.333	1.670	2793	283	2464	6.3	6.7	6.8
30	1.333	1.672	2790	283	2460	6.3	6.7	6.8
60	1.338	1.680	2788	280	2459	6.2	6.7	6.8
90	1.340	1.684	2785	270	2459	6.2	6.7	6.9

The pyrolytic oil obtained was formulated into semisolid dosage forms like gel, ointment and cream for topical application. FTIR compatibility studies of the bio-oil with the selected polymer and other excipients showed no interactions. Physical characterization of the formulations like pH, spread ability, viscosity showed satisfactory results for all three formulations. The stability studies done indicated that prepared formulations were stable. The agar diffusion method concluded that the coconut shell pyrolytic oil and its formulations exhibited good antimicrobial activity against all the tested strains namely *E. coli*, *Bacillus subtilis* and *Candida albicans*. Further studies have to be carried out to prove the safety of the oil. Thus, it could be concluded that bio-oil from biomass can be used effectively in the pharmaceutical industry.

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CONFLICT OF INTEREST

The authors claim no potential conflicts of interest concerning the research, authorship and publication of this article.

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ABBREVIATIONS

MHA: Muller Hinton agar; **SAP:** saponification value; **TLC:** thin layer chromatography; **FTIR:** Fourier transform infrared spectroscopy; **GCMS:** gas

chromatography mass spectrometry; **rpm:** rotation per minute; **g.cm/s:** gram centimeter per second; **°C:** degree celsius; **cP:** centipoise; **M.P:** mobile phase.

Author Contributions

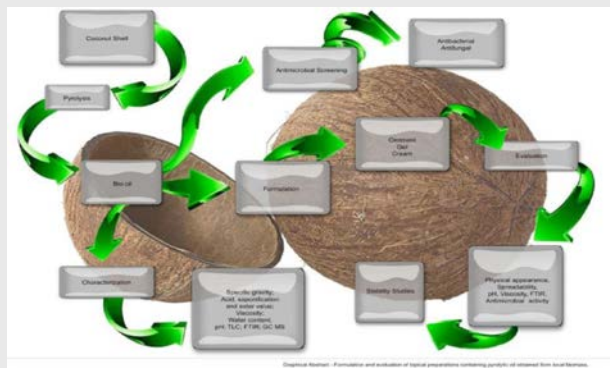
Jithu Jerin James and Sandhya KV conceived and designed the experiments; Jithu Jerin James and Joysa Ruby J performed the experiments; Sandhya KV and Jithu Jerin James analyzed the data; Jithu Jerin James and Sandhya KV wrote the paper.

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PICTORIAL ABSTRACT



SUMMARY

Pyrolytic oil obtained by fast pyrolysis was characterized based on specific gravity, acid/ saponification/ester values, viscosity, water content, pH and found to be acidic. High water content reduced the viscosity significantly. FTIR results showed that pyrolytic oil consists of saturated and unsaturated hydrocarbons; its exact composition was known by GCMS. The pyrolytic oil was formulated into gel, ointment and cream. By FTIR compatibility studies, it was concluded that pyrolytic oil can be used with the selected polymer and excipients. Physical characterization of the formulations like pH, spread ability, viscosity showed satisfactory results for all three formulations. The stability studies done indicated that prepared formulations were stable. The pyrolytic oil and its formulations showed good antibacterial/antifungal activity against *E. coli*, *Bacillus subtilis* and *Candida albicans*. Thus, it could be used for topical formulation as an effective antimicrobial agent.

About Authors



Ms. Jithu Jerin James, working as Assistant Professor in Department of Pharmaceutical Analysis, Acharya & BM Reddy College of Pharmacy, Bangalore – 560107, Karnataka. She has seven years of teaching experience after successful completion of M. Pharm. She has publications in national and international journals and presented papers in international conferences.



Dr. Sandhya K V, currently working as Assistant Professor, Department of Pharmaceutics, FPH, RUAS, Bengaluru. She has published and presented papers in various international journals, international and national conferences. She has worked at Natural Remedies Pvt.Ltd., (R&D), Jubilant Biosys, Bengaluru and at GIIP, Bengaluru. She is a recipient of SRF by ICMR, New Delhi and received grants from VGST & RGUS. Additionally, she holds PG Diploma in Bioinformatics, IPR NLSIU, Bengaluru and is a registered patent agent.



Dr. Joysa Ruby J is currently working as Assistant Professor in Department of Pharmaceutics, Acharya & BM Reddy College of Pharmacy, Bengaluru. She has 13 years of teaching experience and 5 years of Industry experience. She has been published more than 25 research and review papers in various National and International Journals. Her area of research is Nanoparticulate nasal delivery system for targeting various diseases. She also received JRF fellowship from UGC Major Research Project scheme. Additionally she also holds MBA (Human Resource & Marketing) and Diploma in communication from Bharathidasan University.

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