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PREPARATION, CHARACTERIZATION AND DETERMINATION OF ANTIOXIDANT ACTIVITY OF ANTERDHUM PADHATI MASHI (APM) AND BAHIRDHUM PADHATI MASHI (BPM) OF *COCOS NUCIFERA* HUSK

A M Baheti[*1], K. R. Khandelwal[2], Vishnu P. Choudhari[1], Manish S.Wani[1], Ganesh B. Choudhari[1], Shikha A. Baheti[3]

[1]*MAEER's Maharashtra Institute of Pharmacy, Paud Road, Pune, India- 411038.*
[2]*J.S.P.M's RajarshiShahu College of Pharmacy and Research, Tathawde, Pune –33*
[3]*Sohit Pharmacy, Bavdhan, Pune.*

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ABSTRACT

Objective: To prepare and characterise AnterdhumpadhatiMashi (APM) and BahirdhumPadhatiMashi (BPM) of *Cocosnucifera* husk. Methods: The APM and BPM were prepared as per theAyurvedicliterature The physicochemical characterization was performed by Atomic Absorption Spectroscopy, Fourier transform infrared spectroscopy, Powder X-ray diffraction. Antioxidant activity was determined by DPPH assay, H_2O_2 Scavenging Activity, Reducing Power ability. Results: In DSC the region of 80 to 120^{0}C weakening of hydrogen bonds occur with the loss of physically bounded water. This is evident from the weakening of the endothermic peak in both the mashi. An endotherm at 210^{0}C in BPM may be assigned to the condensation product of lignin.PXRD study of confirmed the crystalline form of potassium as potassium chloride sylvite.Peaks at 3647 cm^{-1} and 3456 cm^{-1} are assigned to Stretching vibration mode of the OH$^-$, 2889 cm^{-1}, 2857cm^{-1} indicatesthe aliphatic C-H stretching. The bands between 1480 cm^{-1} and 1300 cm^{-1} may be due to the OH bending vibration which indicates the presence of the phenolic group. The bands around 2400 cm^{-1} denote the presence of C=O stretching indicating that lignin might be rich of methoxy-O-CH_3, C-O-C stretching and C=C stretching (aromatic ring) containing compounds.RESULT: BPM is the powerful free radical scavenger. Conclusion: BPM can be use as an antioxidant agent.The findings of the present research work may be helpful for formulation and standardization of APM and BPM in routine analysis.

Corresponding author
Akshay Motilal Baheti
Associate Professor,
Address:MAEER's Maharashtra Institute of Pharmacy,
MIT campus, Kothrud, Pune 411038, Maharashtra, India
+91-09422060118
akshay.baheti@mippune.edu.in

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INTRODUCTION

Cocosnucifera Linn (Family: Palmae, English: Coconut Palm) is extensively cultivated in southern India and Ceylon. Every Part of the tree is being used for some purpose like food, fuel or timber hence it is called as Kalpravriksha[1].Cocos*nucifera*husk has showed antiviral[2],antimicrobial[2,3],antimalarial[4], antidiarrheal[2],antipyretic[5]antileishmanial[6] and antineoplastic[7].The coconut milk was tested for antiparasitic activity in mice and showed efficacy against *Syphaciaobvelata*, *Aspiculuristetraptera*[8] and *Vampirolepis nana*[9]. Coconut milk is used in renal problems[10] and also used as antidiarrheal[11] and antiulcerogenic[12].infusions made with the coconut inflorescence are used for the oral treatment of menstrual cycle disorders [13]. Ethanolic extract of husk reveled presence of tannins, phenols, leucoanthocyanidins, triterpenes, steroids and flavonoids[14].A butanol extract recovered triterpenes, saponins, and condensed tannins[15]. By chromatographic methods coupled with mass spectroscopy techniques has been demonstrated that drug containscatechin and epicatechin together with condensed tannins[2].

Mashi is the black color, powder dosage form mentioned in Ayurvedic literature[16, 17]. Mashi is a dosage form in which bulk of the raw material is reduced to a greater extent by application of a certain quantum of energy. Due to this treatment, hidden chemical constituent become prominent and/or a new chemical moiety is formed which is therapeutically active. Also due to the thermal degradation or decomposition thermo labile constituents are lost. Thus, without application of any costly method for extraction using anorganic solvent, we can get a therapeutically active organic and inorganic chemical constituent in the form of a black mass known as *Mashi.*According to Ayurvedic literature, *Mashi*can be prepared by two methods viz. *BahirdhumPadhati* (BPM) and *AnterdhumPadhati*(APM). In *BahirdhumPadhati*method (Fig. 1), heating is carried out slowly at about 145-150^0 C, with continuous stirring. In *AnterdhumPadhati* method (Fig. 2), the material of which *Mashi*is to be prepared is packed in between two *SharavSamput*, which are then sealed by sealing clay (*MultaniMatti*). It is then subjected to *Gajaputa* in *GajaputaKund*, which is filled with cow dung cakes. It is then set on fire. When *Gajaputa*becomes *Swangsheet*, *Sharav*is taken out of *Kund*and *Mashi*is collected.
Coconut husk Mashi has been reported to have antiemetic activity in BPM form [16]. Preliminary phytochemical screening revealed that APM and BPM content tannins and flavonoids. Antioxidant activities of polyphenolics derived from plants have claimed beneficial health functions for retarding aging and preventing cancer and cardiovascular diseases[18]. In view of these, our objective was to evaluate the antioxidant activity of BPM and APM in comparison with ascorbic acid through different in vitro models. Literature search suggested that no standardization work has been carried out on Mashi. So we aim to standardize the BPM and APM by modern methods such as Powder X-ray diffraction (PXRD), Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FTIR).

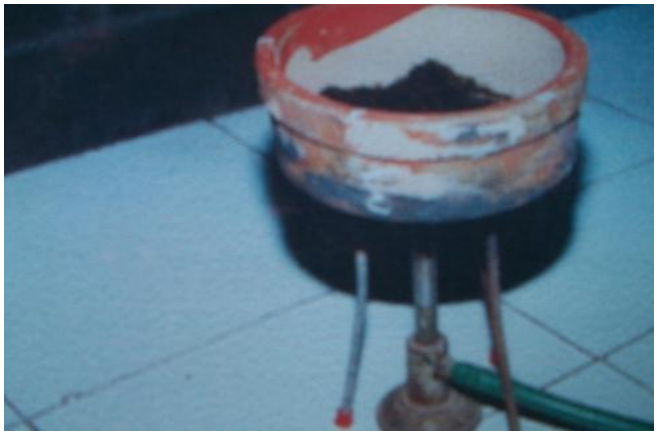

Fig 1: Preparation of bahirdhum padhati mashi.

Fig 2: Preparation of anterdhum padhati mashi.

MATERIALS AND METHODS

Collection of Raw Materials

Cocosnucifera husk was collected by A. M. Baheti from Pune region and identified by Pharmacognosy Department of MAEER's Maharashtra Institute of Pharmacy, Pune. [Voucher specimen (hp/Cocos/01)]

Preparation of Mashi

AnterdhumPadhatiMashi

Husk was packed in between two earthen pots (*Sharavsamput*), which were sealed by *Multtanimatti*. It was subjected to *Gajaputa* (heating into *kund* filled with cow dung cake) in Gajaputakund for 50 min. When Gajaputa became *swangsheet*(cool), *sharav*was taken out of *kund*and Mashi was collected. 100 gm of the husk gave 31.89 gm of APM Mashi

BahirdhumPadhatiMashi

Husk was collected and dried under shade. Coconut husk was heated in an earthen pot at 145-1550C. With continuous stirring till the white fumes ceases to come out. 100 gm of the husk gave 6gm of BPM Mashi (The burnt black powder).

Antioxidant activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay[19]

The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. 0.1mM solution of DPPH in methanol was prepared and 1.0mL of this solution was added to 3.0mL of mashi solution in water at various concentrations (2-1000µg/ml). The mixture was incubated for 45min at room temperature and the absorbance was measured at 517nm against the corresponding blank solution. Ascorbic acid was used as a reference. Percentage inhibition of DPPH free radical was calculated based on the control reading, (which contained DPPH and distilled water without any extract) using the following equation:

$$\text{DPPH Scavenged (\%)} = [(A_c - A_t) / A_c] \times 100$$

Where A_c is the absorbance of the control, and $A_t$is the absorbance of the mashi or standard.
The antioxidant activity was expressed as IC_{50}. The IC_{50} value was defined as the concentration of µg/ml of that mashi that inhibits the formation of DPPH radicals by 50%

Reducing Power Ability[20]

The reducing power ofmashi was determined by the method of Oyaizu. The capacity of extract to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex was determined by recording the absorbance at 700nm after incubation. For this purpose, different concentrations of mashi (2-1000µg/ml) in 1ml of distilled water were mixed with phosphate buffer (2.5ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5mL, 1%). The mixture was incubated at 50˚C for 20 min. Aliquots (2.5 ml) of trichloro acetic acid (TCA, 10%) were added to the mixture. The upper layer of solution (2.5) was mixed with distilled water (2.5mL) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700nm by spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing capability.

H_2O_2 Scavenging Activity[21]

H_2O_2 scavenging activity of mashi was determined according to the method of Ruch et al. A solution of H_2O_2 (40mM) was prepared in phosphate buffer (pH 7.4). 3.4 ml (16-1000µg/ ml) mashi in phosphate buffer were added to solution H_2O_2 (0.6ml, 40Mm). Absorbance was determined at 230nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage ofH_2O_2 scavenging of mashi and ascorbic acid (standard compound) was calculated as:
% Scavenged $H_2O_2 = [(A_c-A_t) / A_c] \times 100$
Where A_c is the absorbance of the control, and A_t is the absorbance of the mashi or standard.

Statistical analysis

All results are expressed as Mean ± Standard deviation (n=3). Student's t-test was carried out to determine significant differences ($P< 0.005$).

Analysis by Modern methods

Fourier Transform Infrared Spectroscopy (FTIR)

The raw material, APM and BPM were subjected for FTIR study. Diffuse reflectance IR spectra were obtained in potassium bromide on Varian 640 IR and expressed in term of wave numbers (cm^{-1}).

Powder X-Ray diffraction (PXRD)

The PXRD patterns were obtained using Cu Ka radiation (λ =1.540 A^0) voltage 40KV, current 40 mA. Data was recorded over a range of 5 to 100^0 at scanning range of 5 X 10^3 C/S using chart speed of 5mm/2θ . (XRD- PW 1729 X-ray generator, PHILIPS; PW 1840 Diffractometer control, PHILIPS, PM 8203 An online recorder, PHILIPS; Software used- UNISTAT, version 6, Megalon Corporation, USA.)

Differential Scanning Calorimetry (DSC)

The DSC (Mettler) thermogram of samples was done by using aluminum crucibles. The system was purged with nitrogen gas (40 ml/ min.) to maintained inert atmosphere.

Atomic Absorption Spectoscopy

Quantitative analysis of coconut husk, APM and BPM was carried out by atomic absorption spectroscopy (CAMO Tech)

RESULTS

Antioxidant activity:

DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay

The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517nm. BPM quenched significantly to DPPH radicals. The APM showed less activity. The free radical scavenging activity increased with increasing concentration.

Table 1 illustrates a significant decrease in DPPH radical concentration due to scavenging ability of mashi of coconut husk and ascorbic acid. The scavenging effect of ascorbic acid, BPM and APM was 92.35 %, 75.54% and 62.35% respectively at concentration 1000 µg/ml. IC_{50} values for ascorbic acid, BPM and APM were 24.62±1.12, 96.01±0.43,256.25±1.32 µg/ml respectively.

Table 1:DPPH scavenging activity of mashiof coconut husk.

Conc.µg/ml	Ascorbic acid	BPM	APM
2	10.21	8.21	7.46
4	13.26	8.52	7.53
16	27.38	15.54	10.23
32	57.72	20.38	16.68
64	86.21	40.71	30.35
128	89.52	51.46	45.24
256	90.2	71.59	59.45
512	90.36	72.35	61.1
1000	92.35	75.54	62.35

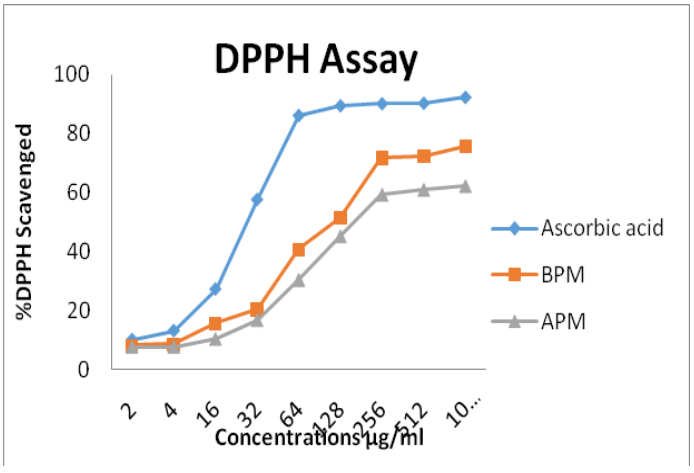

Fig 3: DPPH scavenging power of mashi of coconut husk.

Reducing Power Ability

The reducing power of extracts, mashi of unripe coconut husk and ascorbic acid is shown in Table 2. The reducing power increased with the increase in the concentration of mashi.

Table 2: Reducing power ability of mashi of coconut husk.

Conc. µg/ml	Ascorbic acid	BPM	APM
2	0.085	0.065	0.052
4	0.096	0.075	0.065
16	0.113	0.100	0.096
32	0.116	0.112	0.111
64	0.233	0.116	0.112
128	0.412	0.212	0.118
256	0.476	0.239	0.211
512	0.786	0.587	0.456
1000	0.909	0.754	0.652

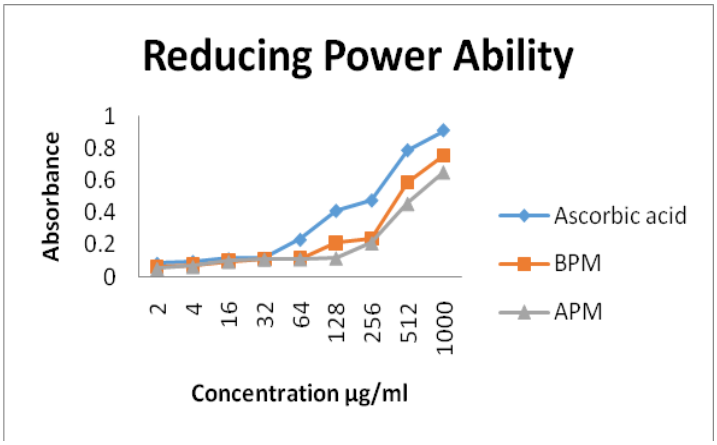

Fig 4: Reducing power ability of mashi of coconut husk.

H_2O_2Scavenging Activity

Table 3 illustrates the decrease in hydrogen peroxide free radicals due to scavenging activity of mashi of coconut husk. At 1000 µg/ml of ascorbic acid, BPM, APM exhibited 78.568±1.25, 72.364±1.11, 59.322±1.36respectively. Ascorbic acid and BPM effectively scavenged the hydrogen peroxide radicals in dose-dependent manner. IC_{50} values for ascorbic acid, BPM, APM were 26.32±0.35 µg/ml, 24.35±0.76 µg/ml,194.25±0.81 µg/ml respectively. The activity of APM was very less compare to BPM

Table 3:H_2O_2 scavenging activity of mashi of coconut husk.

Conc. µg/ml	Ascorbic acid	BPM	APM
16	39.852	39.532	22.125
32	52.247	54.214	32.122
64	62.924	60.452	41.339
128	68.954	68.214	49.287
256	73.548	72.251	53.215
512	78.568	72.364	59.322
1000	80.321	75.225	62.118

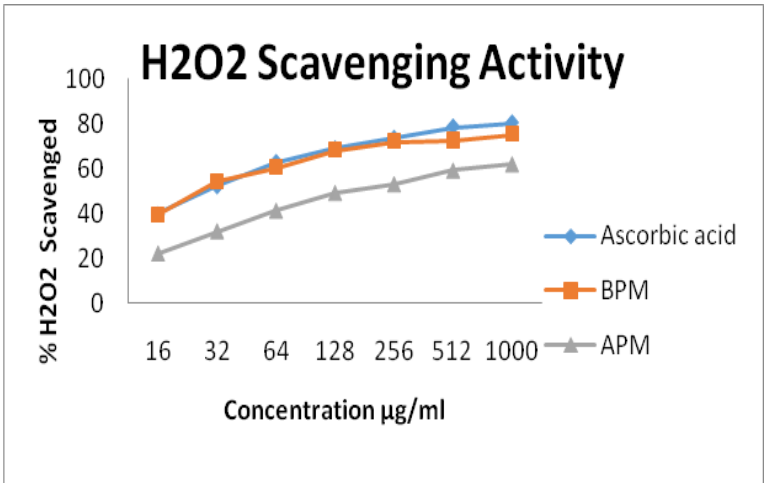

Fig 5:H_2O_2 scavenging activity of mashi of coconut husk.

Analysis by Modern Methods

Differential Scanning Calorimetry (DSC)

The BPM showed two endothermic peaks, while APM showed only one endothermic peak. Results are presented in Fig 6-7, Table:4

Table 4:DSC data of (A): APM and (B) :BPM.

Transition No	Normalized Energy (J/g)		Reaction		Temperature (^{0}C) onset		Peak		Endset	
	A	B	A	B	A	B	A	B	A	B
1	-33.69	-14.07	Endo	Endo	98.06	93.43	103.30	97.05	111.01	101.83
2	--	-9.29	--	Endo	--	110.14	--	117.09	--	123.30

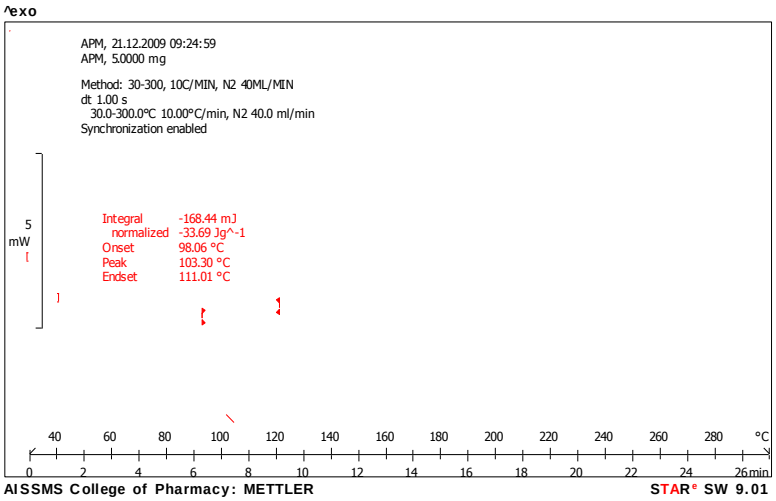

Fig 6: DSC pattern of APM.

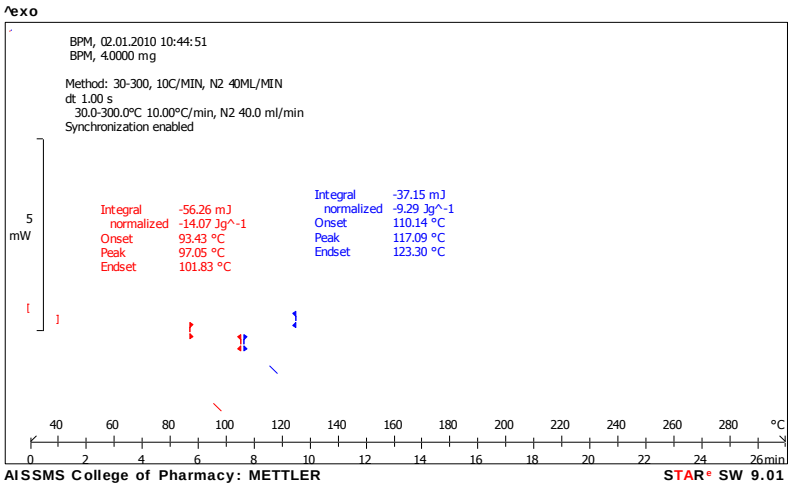

Fig 7: DSC pattern of BPM.

Powder X-Ray Diffraction (PXRD) of Mashi

PXRD patterns of anterdhumpadhatimashi and bahirdhumpadhatimashi showed crystallinity. The crystallinity of bahirdhumpadhatimashi was found more than the anterdhumpadhatimashi. Both mashi showed the same pattern with the difference in peak heights. D values of peaks of both mashi were very close to those of potassium chloride Sylvite crystals. Which suggest the presence of potassium chloride sylvite in both the mashi. The differences in the diffraction patterns is shown in Fig 8-9.

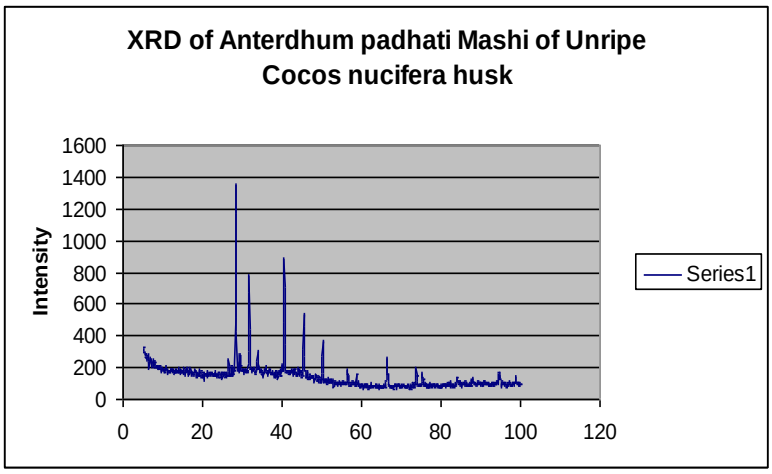

Fig 8: XRD of APM.

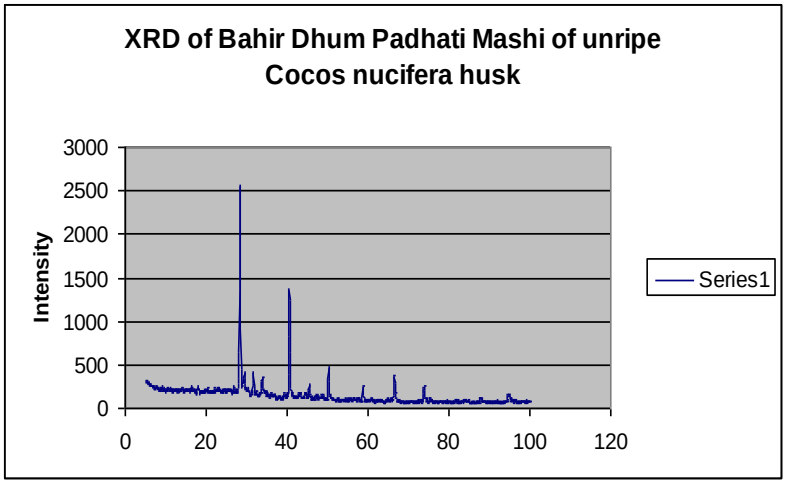

Fig 9: XRD of BPM.

Fourier Transform Infrared Spectroscopy (FTIR) of APM and BPM

APM and BPM showed a similar pattern of the spectra. Peaks at 3624 cm^{-1} and 3456 cm^{-1} are assigned to Stretching vibration mode of the OH^-, 2889 cm^{-1} indicatesthe aliphatic C-H stretching, The presence of bands around 2400 cm^{-1} denotes the presence of C=O stretching, The bands between 1480 cm^{-1} and 1300 cm^{-1} may be due to the presence of the OH bending vibration which indicates the presence of phenolic group. FTIR spectra is presented in Fig 10-11

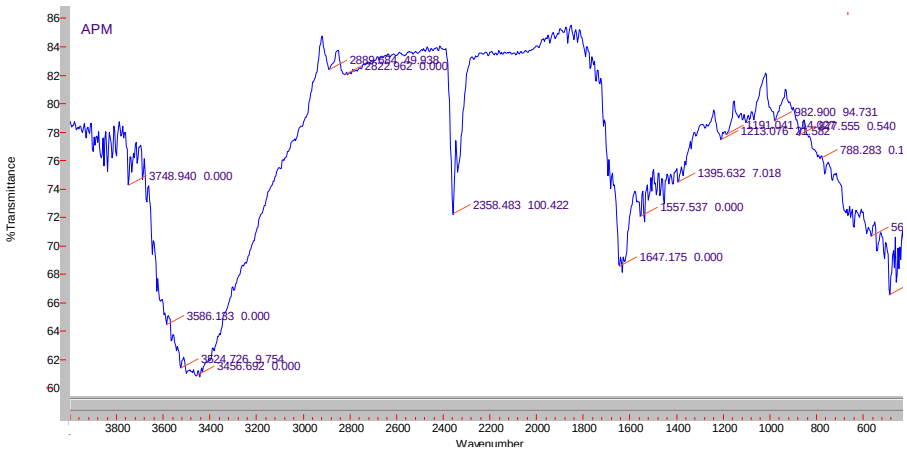

Fig 10: FTIR spectra of APM.

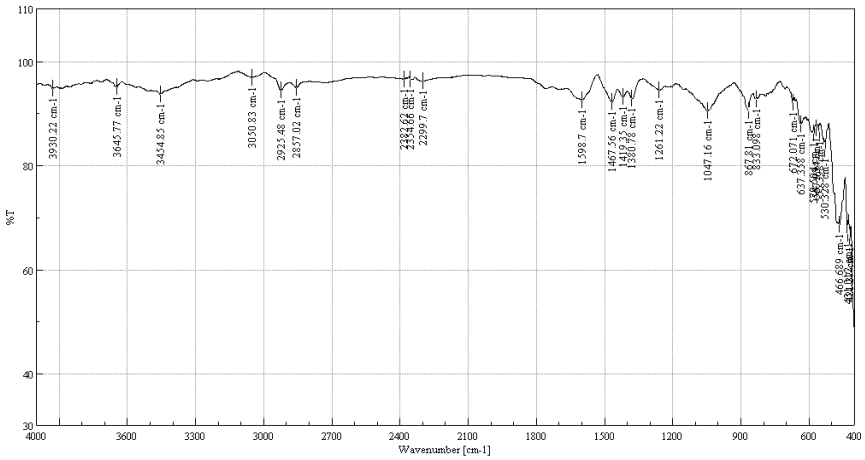

Fig 11: FTIR spectra of BPM.

Atomic Absorption Spectroscopy

Quantitative analysis of coconut husk and mashiwas carried out by Atomic absorption spectrometry. .Results are presented in Table 5.

Table 5 :Quantitative analysis of coconut husk and mashi.

Sr. No	Inorganic radicals	Estimated % of APM	Estimated % of BPM	Estimated % of coconut husk
1	Sodium	0.89	6.75	0.02
2	Potassium	1.651	12.358	1.087
3	Chloride	Nil	2.923	0.53
4	Lead	0.004	0.0028	Nil
5	Arsenic	Nil	Nil	Nil
6	Carbonate	0.768	7.0325	0.32

DISCUSSION

Comparative study of the APM and BPM revealed a major difference in yield. The yield of APM was more than the BPM. APM had poor flow ability, lower solubility compares to BPM. Inorganic content determination confirmed that there is the only quantitative difference in the inorganic constituents and not qualitative. Comparison of the yield of the mashi and its inorganic content estimation confirms that quantitative difference found is dependent on the yield. More the yield, less the inorganic content and vice versa. Hence inorganic content of BPM was more than APM. Potassium was found as 12.358 % in BPM and it was 1.651% in APM and 1.087%in fiber.

DSC thermogram of APM and BPM was reproducible. Therefore it may be considered as a promising tool for quality control. In the region of 80 to 120^0C weakening of hydrogen bonds occur with the loss of physically bounded water. This is evident from the weaking of the endothermic peak in both the mashi. An endotherm at 210^0C in BPM may be assigned to the condensation product of lignin. It is reported that lignin is devitrified by condensing and softening process in temperature 135-250^0C.

PXRD study of the APM and BPM confirmed the crystalline form of potassium as potassium chloride sylvite, which is a very common form of potassium salt occurring in various natural products.

APM and BPM showed a similar pattern of the FTIR spectra. Peaks at 3647 cm^{-1} and 3456 cm^{-1} are assigned to Stretching vibration mode of the OH^-, 2889 cm^{-1}, 2857cm^{-1} indicatesthe aliphatic C-H stretching. The bands between 1480 cm^{-1} and 1300 cm^{-1} may be due to the presence of the OH bending vibration which indicates the presence of phenolic group. The presence of bands around 2400 cm^{-1} denotes the presence of C=O stretching indicating that lignin might be rich of methoxy-O-CH_3, C-O-C stretching and C=C stretching (aromatic ring) containing compounds.

The results of DPPH activity, reducing power activity and H_2O_2 scavenging activity clearly indicated that BPM is powerful free radical scavenger. Scavenging of hydrogen peroxide which may be attributed to the presence of the phenolic groups that might be donating electrons to hydrogen peroxide, and neutralizing it.

CONCLUSION

It can be used as an antioxidant. It may be concluded that modern analytical techniques may be successfully integrated into these studies for better quality control and to evaluate the quality aspects of mashi. The findings of the present research work may be helpful for further formulation and standardization of APM and BPM in routine analysis.
Further in vivo antioxidant activities are required to investigate

CONFLICT OF THE INTREST

Declared none

LIST OF ABBREVATIONS

APM	:	Anterdhum Padhati Mashi
BPM	:	Bahirdhum Padhati Mashi
PXRD	:	Powder X-ray diffraction
DSC	:	.Differential Scanning Calorimetry
FTIR	:	Fourier Transform Infrared Spectroscopy

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