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Potential Greener Protocol for Vanillin Production from Oxidative Depolymerisation of Sawdust Lignin using Recyclable [OMIM][OH] and Evaluation of Its Antioxidant Power by FRAP Assay

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Abstract

In this work, we have used sawdust, a waste biomaterial of wood working operations for the isolation of lignin in ionic liquids, without using any catalyst followed by production of vanillin from oxidative depolymerization of lignin in the ionic liquid media using oxygen. Out of the three ionic liquids used in our work, the highest lignin and vanillin yields were obtained for [OMIM][OH], followed by [BMIM][OH]. Both the lignin solubility and vanillin production were favoured by high temperature conditions and longer reaction time in [OMIM][OH]. The amount of isolated lignin was improved with the increase in concentration of the ionic liquid. However, the amount of [OMIM][OH] did not seem to affect the amount of vanillin produced. UV-Vis., FT-IR, mass spectroscopy, ^{1}H-NMR and ^{13}C-NMR spectroscopy were used to characterize the vanillin produced by our method. The antioxidant activity of the produced vanillin was evaluated by FRAP Assay and found that antioxidant activity of the produced vanillin is analogous to that of standard pure vanillin.

***Key words*:** Lignin, Vanillin, Isolation, Oxidation, Waste biomaterial, Recyclable, Sawdust, Ionic liquid

Vanilla, being the world's most admired flavouring material, has comprehensive applications in food, beverages, preservatives, liquors, perfumery, as well as in pharmaceutical industry [1]. The diverse properties of Vanilla are accredited to its chief constituent vanillin (4-hydroxy-3-methoxybenzaldehyde), a white coloured crystalline powder with a pleasant, sweet, and strong aroma, which occurs with concentration of around 1.0-2.0% w/w in cured pods of Vanilla orchid, e.g., *Vanillus planifolia* and *Vanillus tahitensis* [2-3]. More importantly, vanillin is found to have a range of significant medicinal properties like anti-sickle cell anaemia activity, anti-mutagen, antioxidant, anticarcinogenic, anti-hepatotoxic, anti-hyperlipidemic, antiviral, anti-inflammatory, antiseptic, anti-microbial, analgesic, anaesthetic, anti-aggregate etc., and can also be used as antifoaming agent, mosquito preventing agent, sleep prevention agent, aphrodisiac, vulcanization inhibitor, natural preservative to prevent food spoilage, and as a chemical precursor for pharmaceutical and agrochemicals industries [4-9]. But, due to the existence of very less amount of vanillin in Vanilla orchid, it can cover just 0.2% of the market place necessity [10] and therefore, there is a requirement of production of vanillin from other sources to fulfil its market place requirement. Vanillin can also be obtained by chemical synthesis [11] from various sources like from eugenol present in clove oil, lignin-containing sulfite liquor - which is obtained in wood pulping in paper manufacturing process, through oxidation of lignin present in hardwood, softwood or any other lignin sources [12-15]. Different methods like oxidation, hydrolysis or pyrolysis can be used to break down lignin into low molecular weight phenolic compounds with low percentage yields. It was reported in numerous works that mild oxidation of lignin in an alkaline medium can produce high yields of vanillin and syringaldehyde [16]. Alternatively, lignin can be isolated to a great extent from lignocellulosic biomass via strong dilute acid or alkaline treatment, organosolvent process or sulphite pulping method [17]. The procedure of isolation of lignocellulosic biomass by means of the Kraft method or sulphate method use concentrated and strong bases, particularly a combination of sodium hydroxide and sodium sulphide, recognised as white liquor [18]. After processing the lignocellulosic biomass, black liquor is obtained which contains lignin fragments and is utilized for the vanillin production via oxidation [16]. However, supplementary steps are required even after this, for the neutralization of the black liquor with high pH, to achieve the recovered vanillin. This method is highly corrosive, hazardous and detrimental to the environment [19] and no longer accepted. In addition, Organosolvent process was reported to be useful for lignin isolation. Nevertheless, because of highly volatile, highly flammable, and toxic organic solvents this process is not widely

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accepted. Also, it typically involves high pressure condition and advanced equipment for lignin oxidation due to the lower boiling point of various organic solvents with high vapour pressure [20]. Along with all these, not any of the commonly used isolation or oxidation processes that have been so far reported facilitate the production of lignin or vanillin.

Thus, it is gradually becoming more crucial and appropriate to substitute environmentally detrimental volatile organic solvents in various chemical reactions and manufacturing processes and to replace them with alternative greener solvents like ionic liquids. Ionic liquids are recognised as "Designer Solvents" in modern times as there is a possibility to design them just by altering the cation or anion. They are called 'Green solvents' due to the existence of attractive properties like negligible vapour pressure (non-volatility), superior ionic conductivity, high chemical and thermal stability, non-flammable nature, non toxicity, recyclability, easy preparation and product recovery, no severe side reactions and they can afford superior solubility for wide-ranging organic and inorganic compounds. As they are proficient in dissolving and blending biopolymers with high efficiency and extend a prospectively safer, cleaner, uncontaminated medium to carry out chemical reactions, they are acknowledged as aspiring, lucrative and novel substitutes for organic solvents in case of lignocellulosic materials and synthetic organic chemistry [21-26].

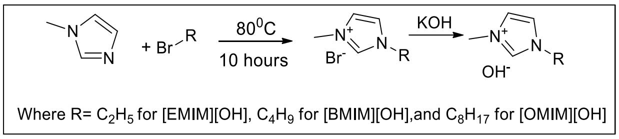
In this study, sawdust was used as the raw material and three different basic ionic liquids, viz., [EMIM] [OH], [BMIM] [OH] and [OMIM] [OH] were used for the lignin isolation, without any solvent, catalyst or external base and yields were compared to conventional methods. Then, we examined oxidative production of vanillin from the lignin in the ionic liquid medium by means of oxygen. The vanillin produced from oxidative depolymerization of sawdust lignin was characterized by different spectroscopic analyses. The total antioxidant potential of the produced vanillin was evaluated by FRAP assay and the results were compared with standard pure vanillin to know the effectiveness of the produced vanillin as a potential antioxidant.

MATERIALS AND METHODS

Sawdust used in the experiment was supplied by local sawmill in Dibrugarh district of Assam that specially deals with softwoods for various construction purposes. 1-methyl imidazole, n-ethyl bromide, n-butyl bromide, n-octyl bromide, TPTZ, acetic acid, sulphuric acid and hydrochloric acid were bought from Sigma Aldrich. Standard vanillin was purchased from TCI. Methanol (LR grade), acetone, diethyl ether, sodium acetate trihydrate, ethyl acetate, sodium hydroxide and potassium hydroxide were bought from Merck. Ascorbic acid was purchased from Acros-organic and $FeCl_3 \cdot 6H_2O$ was purchased from Rankem.

Preparation of ionic liquids

For the preparation of [OMIM] [OH], 1-methyl imidazole (0.8 mol) and n-octyl bromide (0.8 mol) were taken together and stirred well at 80°C for 10 hours in neat condition in a round bottom flask. After that, the white solid [OMIM] [Br], accordingly formed, was cleansed using ethyl acetate for four times to completely remove n-octyl bromide and other impurities that may possibly present. Then it was put through vacuum to achieve the pure solid [OMIM] [Br]. Afterwards, it was treated with potassium hydroxide (0.8 mol), in acetone, stirred well for the night at room temperature and then filtered. Removal of acetone was completed by subjecting it to vacuum and finally, pure dark brown [OMIM] [OH] was obtained. Other two ionic liquids, [BMIM][OH] and [EMIM][OH] were also obtained in a analogous method by using n-butyl bromide and ethyl bromide respectively, instead of n-octyl bromide. The general reaction is represented in (Scheme 1).



Scheme 1 General scheme for the synthesis of ionic liquids

Isolation of lignin from sawdust using ionic liquid media

We followed the method described by Shamsuri and Abdullah for the isolation process of lignin from sawdust by in an ionic liquid medium with minor alteration [27]. For the isolation process, the sawdust was crushed and kept in an oven at 100 °C for a night to get rid of the moisture present. Then, an oven dry weight of 5.0 g of wood dust was taken in a round bottom flask with 0.05 moles of the ionic liquids in the flask and the contents in the glass flask was stirred with 470 rpm at a temperature of 25 °C for 1 hour. The amount of the ionic liquids was changed to 0.06, 0.07, 0.08, 0.09 and 0.1 moles separately to find out the optimum amount of the ionic liquids for the isolation process. The time of the isolation process was also changed to 1, 2, 3, 4, 5 and 6 hours individually to have the higher yields of lignin. After that, the insoluble components present in the reaction mixture were removed by filtration from the subsequent solution whereas the soluble lignin was precipitated by addition of small amount of methanol and the precipitated residue was washed and filtered off upon completion. The isolated lignin was washed using distilled water for quite a lot of times in order to remove any ionic liquid present in the residue. After the completion of the filtration process, the lignin was collected and thoroughly dehydrated using an oven at around 80 °C for more than 24 hours until dryness and weighted. The temperature for isolation was also optimised by changing the temperature to 40, 60, 80, 100, 120, 140 and finally to 160 °C by immersing in sand bath independently. The isolation processes were carried out in a repetitive manner for five times to have enough lignin for the oxidation process and the dried isolated lignin is kept in a desiccator before further use after calculating their average yields.

Isolation of lignin from sawdust in acidic medium

Isolation of lignin using acidic medium was done in accordance with the process illustrated by Pouteaua *et al*. [28] with trivial variation. 5.0 g of the dried sawdust was mixed with a combination of acetic acid and 72% sulphuric acid in a round bottom flask with sawdust to solvent ratio of 1:20 (w/w) and it was heated for 3 hours under reflux and filtered. Subsequently lignin washed using distilled water, after the removal of the solvent, to make it completely neutral. The isolated lignin was then dried using an oven at 80 °C for a period of almost 24 hours to complete dryness and then kept in a desiccator after calculating the average yield.

Isolation of lignin from sawdust in alkaline medium

For the lignin isolation process in alkaline medium, the procedure described by Jiebing *et al*. [29] with minor modification was used. 5.0g of sawdust was taken in a flask containing 1.5 mol/L sodium hydroxide solution and heated at 70 °C for 3 hours with a 1:10 sawdust to solvent ratio, by weight. Then the mixture was filtered and the solid impurities were cleaned out. The resulting black liquor lignin was then acidified to pH 4 by adding necessary amount of sulfuric acid. The resulting precipitate was filtered and then suspended in water and then filtered again. The purified lignin, thus obtained, was dried in an oven at 40 °C for around 24 hours to achieve complete dryness, kept in a desiccator before further use after calculation of the average yield.

Production of vanillin from oxidative depolymerization of isolated lignin using ionic liquid

2g of the isolated lignin was taken in a beaker and dissolved in 0.01 moles the ionic liquids. After that, the solution was transferred into a round-bottom flask, with a magnetic stirrer and oxygen gas was passed for 1 hour through the flask in order to oxidize the lignin at 25 °C. The oxidative mixture was then mixed with 20 ml of diethyl ether; kept for 30 minutes and then filtered. Then, the filtrate was transferred to a separating funnel in order to extract the product and to separate any un-oxidized lignin present. The solution formed at the bottom of the funnel was collected and the pure vanillin was separated by using column chromatography. The amount of ionic liquid [OMIM][OH] was increased to 0.02, 0.03, 0.04, 0.05 and 0.06 separately to find out the maximum concentration of ionic liquids required for the highest yield of vanillin. The time of passing the oxygen gas through the flask was also increased to 2, 3, 4, 5 and 6 hours to find out the optimum time period for the oxidative production of vanillin as well as, the temperature is increased from 25 °C to 40, 60, 80, 100, 120, 140, 160 °C separately to find out the optimum temperature. Oxidation processes were also completed in a repetitive manner for five times with the optimum conditions and the average yields of vanillin were calculated and used for characterization and further experiments [27].

Determination of antioxidant potential of produced vanillin by FRAP Assay

The FRAP reagent was prepared by incubating a mixture of tripyridyltriazine (TPTZ) solution, $FeCl_3•6H_2O$, and acetate buffer (pH 3.6) in a 1:1:10 ratio at 37°C for 5 min. Thereafter, a portion of ferric tripyridyltriazine (Fe^{3+}-TPTZ) mixture and solution of different concentration of standard vanillin available in the laboratory, produced vanillin from sawdust lignin and $FeSO_4·7H_2O$ (taken as standard) solution (20, 40, 60, 80, 100 µM) were adjusted to 1000µl, separately, by adding de-ionized water and the mixtures were incubated for 30 minutes at 37 °C. Blank absorbance values were recorded for each concentration of all the three samples at 593 nm [30-31] before the addition of commercially available vanillin and produced vanillin to the FRAP solution and thereafter absorbance values were recorded in the UV-visible spectrophotometer after adding the vanillin solution or ferrous sulphate solution. After that, the absorbance verses vanillin concentration graphs were plotted and the FRAP values of vanillin were determined by the following formula:

$$\text{FRAP value } (\mu M) = (\text{Absorbance of vanillin solution} / \text{absorbance of standard } Fe^{2+} \text{ solution}) \times Fe^{2+} \text{ conc. } (\mu M)$$

RESULTS AND DISCUSSION

Optimum condition for isolation of sawdust lignin using ionic liquid medium

The weight of our isolated lignin by using different ionic liquids and conventional processes were measured after oven-drying at 85 °C for 24 hours. Out of the three ionic liquids, [OMIM][OH] was found to give best results followed by [BMIM][OH]. The isolation condition for maximum lignin solubility in [OMIM][OH] was optimized by varying its concentration and as the concentration of ionic liquid increases from 0.05 moles to 0.1 moles at 25 °C for 1 hour, the amount of soluble lignin in [OMIM][OH] increased from 0.128g to 0.308g with maximum lignin yield at 0.1 moles of the ionic liquid. So, it was established from our study (Table 1) that, higher ionic liquid concentration augments the solubility of sawdust due to decrease in the quantity of lignin saturation.

Table 1 Yields of isolated lignin using different amounts of ionic liquids

Isolation medium	Time period of isolation	Ionic liquid amount (moles)	lignin yield (wt.% of the sawdust used)
[EMIM][OH]	1 hour	0.05	0.96
		0.06	1.20
		0.07	1.98
		0.08	2.34
		0.09	3.04
		0.1	3.57
[BMIM][OH]	1 hour	0.05	1.80
		0.06	2.46
		0.07	2.87
		0.08	3.20
		0.09	3.87
		0.1	4.56
[OMIM][OH]	1 hour	0.05	2.57
		0.06	3.23
		0.07	4.15
		0.08	5.07
		0.09	5.95
		0.1	6.17

Table 2 Optimisation of isolation duration using 0.1 moles of [OMIM][OH]

Reaction time (hours)	Lignin yield (wt.% of the sawdust used)
1	6.17
2	7.23
3	8.34
4	9.37
5	9.92
6	9.94
7	9.95

Table 3 Optimisation of isolation temperature for 0.1 moles of [OMIM][OH] for a period of 5hrs

Isolation temperature (°C)	Lignin yield (wt.% of the Sawdust used)
25°C	9.96
40°C	11.57
60°C	12.68
80°C	13.73
100°C	14.80
120°C	15.98
140°C	17.03

We have also optimized the time period of lignin isolation and found that by increasing the duration of isolation, yields of soluble lignin can be increased (Table 2). We found the highest yield of soluble lignin (0.496g) for duration of 5 hours at 25 °C. After 5 hours, much differences were not observed in the yields of isolated lignin even after prolong durations. Thus, it was observed that under suitable condition, the release of lignin from the wood cell walls increases with increase in the time period of isolation. Moreover, the influence of temperature on optimised reactions conditions were also examined by changing the temperatures and observed that higher temperature enhances the lignin solubility (Table 3). For 0.1 moles of [OMIM][OH], and 5 hours of isolation period, solubility of lignin was found to be increased from 0.496g (9.92%) at 25 °C to 0.0.741g (14.83%) at 100 °C with a maximum value of 0.851g (17.03%) for 140 °C. Further increase in the temperature does not seem to affect the yields of isolated lignin to a greater extent. These results indicate that, high temperature may augment the lignin solubility in the ionic liquid by increasing the interaction between the lignin and ionic liquid. It was observed that in the isolation process using 0.5 moles [OMIM][OH], at a temperature of 100 °C for 5 hours, 17.03wt.% lignin from the sawdust could be isolated, whereas, isolation in acidic and alkaline medium yielded 21.14wt.% and 18.69wt.% lignin.

Table 4 Yield of lignin isolated from sawdust using [OMIM][OH], acidic and alkaline medium

	Ionic liquid isolation (Using [OMIM][OH] under the optimum conditions)	Acidic isolation	Alkaline isolation
Yield of isolated lignin from sawdust (wt.% of the sawdust used initially)	17.03	21.14 (Impurities present)	18.69 (Impurities present)

Thus, as displayed in (Table 4), it was established that conventional processes utilizing acid and alkaline medium resulted in a better yield of lignin isolation from sawdust under strong conditions compared to our ionic liquid medium. However, the precipitated lignin obtained from liquors recognised as acid-soluble and alkali-soluble lignin cannot be considered to be pure or impurity free as non-homogenous appearance of liquors were observed even after centrifugation. So, there is a probability of presence of comparatively higher quantities of other modified compounds, which resulted in higher yields. Furthermore, due to the presence of impurities, there is possibility of additional contamination resulting in inaccuracy in the estimation process.

Table 5 Optimization of oxidation duration using [OMIM][OH] at temperature 25°C

Temperature	Oxidation duration (hours)	Vanillin yield (wt.% of the initial lignin)
25°C	2hrs	1.05
	4hrs	2.38
	6hrs	3.68
	8hrs	4.75
	10hrs	6.26
	12 hrs	6.27
	14 hrs	6.29

Optimum conditions for oxidative depolymerisation of lignin to vanillin

During the oxidation process, lignin goes through depolymerisation and the depolymerisation rate is reliant on the used for oxidation conditions. Throughout the dissolution process of lignin in the ionic liquid, the primary chemical structure of lignin is not distorted. However, as soon as the vanillin formation occurs, there is a change in primary structure of lignin. It was observed that, the rate of oxidative production of vanillin was not found to be altered by changing the concentration of [OMIM][OH], whereas, the yield of vanillin (wt.% of the lignin used for oxidation) was found to be increased from 1.05% (0.0210g) for 2 hours oxidation period to 6.26% (0.1252 gm) for a period of 10 hours, indicating that the time period for the reaction must be maximised to augment the depolymerisation process of lignin (Table 5). Increase in time period to a greater extent did not lead to the amplification of the amount of vanillin. Also, it was observed that the amount of vanillin for 10 hours oxidation was increased from 6.26% (0.1252 gm) at 25 °C to 21.56% (0.4312 gm) at 100 °C and 32.89% (0.6578 gm) at a temperature of 140 °C, as displayed in (Table 6). Also, the amount of vanillin did not seem to be increased when the temperature is increased to 160 °C or more. This indicates that high temperature condition augments the oxygen solubility in the [OMIM][OH] and increases the rate of lignin depolymerisation. The product acquired from sawdust lignin degradation in our study following the optimum conditions was found to release a fragrance indistinguishable to the standard vanillin available in our laboratory.

Table 6 Optimization of oxidation temperature using [OMIM][OH] in a duration of 10 hours

Oxidation time	Temperature	Vanillin yield (wt.% of initial lignin)
10 hours	25°C	6.26
	40°C	9.54
	60°C	13.36
	80°C	17.63
	100°C	21.56
	120°C	26.56
	140°C	32.89

Recyclability of [OMIM][OH] for isolation and oxidation processes

The ionic liquid [OMIM][OH] was recycled up-to four times for both the lignin isolation process from sawdust as well as for the oxidative depolymerisation of lignin to vanillin without much loss of their production yields and the results are mentioned in (Table 7).

Table 7 Yields of isolated lignin from sawdust and vanillin produced up-to four cycles in [OMIM][OH]

Product	First cycle (Yield in wt. %)	Second cycle (Yield in wt. %)	Third cycle (Yield in wt. %)	Fourth cycle (Yield in wt. %)
Lignin isolated from sawdust (Weight % of the sawdust used initially)	17.03	15.06	11.46	9.24
Vanillin (wt.% of the initial lignin)	32.89	30.18	28.06	24.03

Characterization of vanillin produced by oxidation of sawdust lignin using [OMIM][OH]

The production of vanillin was monitored by TLC and the identity of the compound is confirmed by FT-IR, UV-vis., mass spectral and NMR analyses.

Infrared spectrum

The infrared spectrum of the vanillin produced by oxidative depolymerization of sawdust lignin using [OMIM][OH] was recorded (Fig 1) within the range 400-4000 cm^{-1} by means of a Shimadzu FT-IR spectrophotometer, Model:

Prestige 21. In the FT-IR spectrum, a broad band at around 3200 cm^{-1} designates the existence of a O-H stretching of phenolic hydroxyl group and a sharp peak at 1666 cm^{-1} reveals C=O stretching of an aldehyde group. Along with these, sharp peaks at 1286 cm^{-1} as well as at 1030 cm^{-1} signify the existence of C-O stretching of ether and C-O stretching of phenolic group (Table 8).

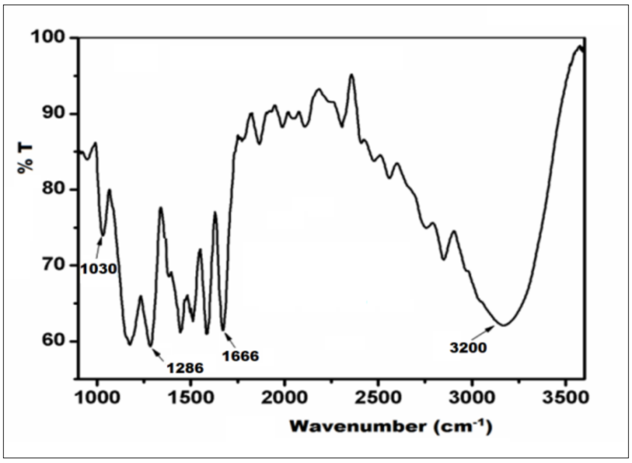


Fig 1 FT-IR spectrum of vanillin produced by oxidative production from sawdust lignin

Table 8 FTIR bands of the vanillin produced by oxidation of sawdust lignin using [OMIM][OH]

FTIR Bands	Band origin	Functional group
3200 cm^{-1}	O-H stretching	Phenol
1666 cm^{-1}	C=O stretching	Aldehyde
1286 cm^{-1}	C-O stretching	Ether
1030 cm^{-1}	C-O stretching	Phenol

UV-visible spectrum

The electronic spectrum of the compound produced by oxidation of sawdust lignin (Fig 2) was recorded using methanol within the range of 200-900 nm using Shimadzu, UV-Vis. Spectrophotometer: Model UV-1700 and absorptions were observed at 307 nm and 274 nm owing to n → π* and π → π* electronic transitions correspondingly. The n → π* transition is observed owing to the participation of carbonyl group of the aldehyde. The values of the electronic spectrum authenticated the formation of vanillin as these absorption values are in concordance with the formerly reported values of vanillin [32].

Mass spectrum

The identity of the vanillin produced from oxidation of lignin by our study was also established from its mass spectrum using Agilent GC-7820A/MS5975 analyzer. An m/z value of 152.1 for the molecular ion, [M^+] of the compound formed after our study (Fig 3) also affirms the formation of vanillin.

^{1}H- NMR and ^{13}C-NMR Spectra

The ^{1}H-NMR and ^{13}C-NMR spectra of vanillin were recorded in DMSO-d_6 by JEOL, 400 MHz FT-NMR spectrophotometer at 298 K using TMS as the internal standard. The chemical shifts at 9.852, 5.081 and 3.782 ppm in the ^{1}H-NMR spectrum of the produced vanillin indicates the existance of aldehyde, phenolic and methoxy protons, respectively and the peaks at 7.313-6.795 ppm indicates other aromatic protons (Fig 4). Whereas, in its ^{13}C-NMR spectrum, the occurance of chemical shifts at 193.10 and 57.71 ppm indicates existance of aldehyde and methoxy carbons and the chemical shifts at 154.99, 149.67 and 131.66 indicates the aromatic carbons linked to the methoxy, hydroxyl and aldehyde group, respectively, as displayed in (Fig 5). Thus, from all these spectral analyses, we can confidently claim that the compound produced from the oxidative depolymerisation of isolated lignin from sawdust by our work using [OMIM][OH], is confirmed to be vanillin.

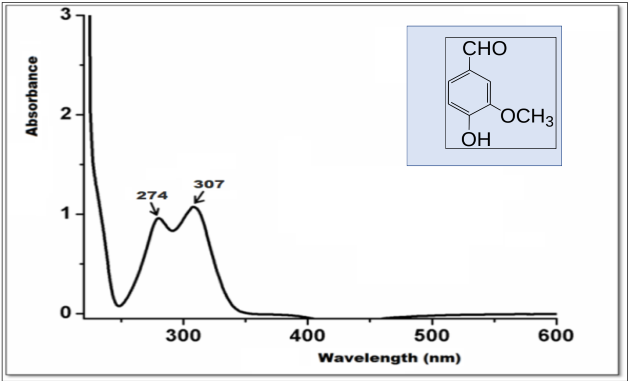


Fig 2 UV-visible spectrum of vanillin produced from sawdust lignin

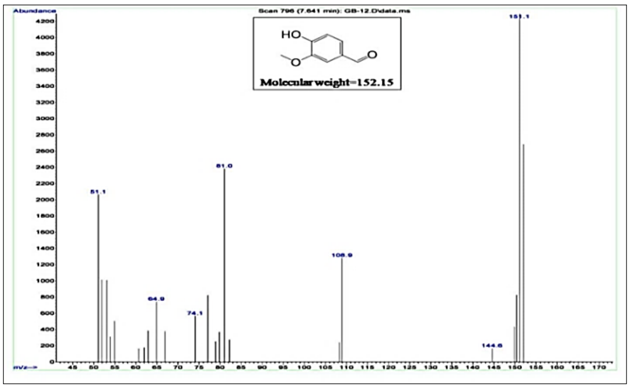


Fig 3 Mass spectrum of the vanillin produced by oxidative depolymerisation of sawdust lignin

Table 9 Absorbance and FRAP values of ascorbic acid, standard vanillin and produced vanillin

Compound	Conc. (μmol/L)	Absorbance Fe^{2+} solution 593nm	Absorbance at 593nm	FRAP values (μmol/L)	Mean ± SD (Standard Vanillin)
Standard vanillin	20	0.094	0.498	105.957	299.455±156
	40	0.102	0.512	200.784	
	60	0.125	0.596	286.08	
	80	0.136	0.698	410.588	
	100	0.147	0.726	493.877	
Produced vanillin	20	0.094	0.486	103.404	293.459±152
	40	0.102	0.506	198.431	
	60	0.125	0.583	279.84	
	80	0.136	0.681	400.588	
	100	0.147	0.713	485.034	

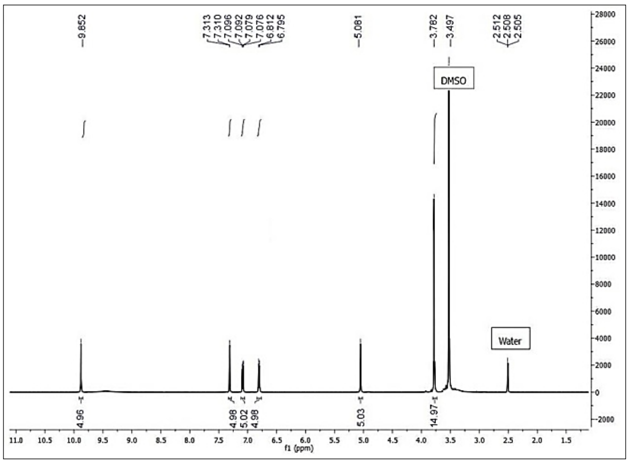


Fig 4 ^{1}H-NMR spectra of our produced vanillin

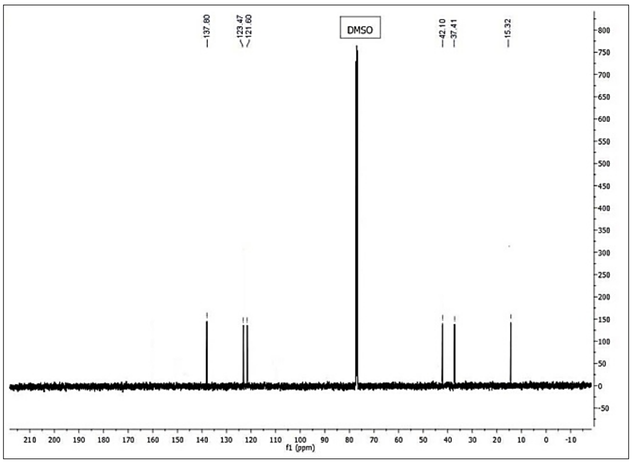


Fig 5 ^{13}C-NMR spectra of our produced vanillin

Determination of antioxidant power by FRAP assay

The observations found by employing the Ferric Reducing Antioxidant Power for both the standard vanillin and produced vanillin in our experiment are expressed in (Fig 6, Table 9).

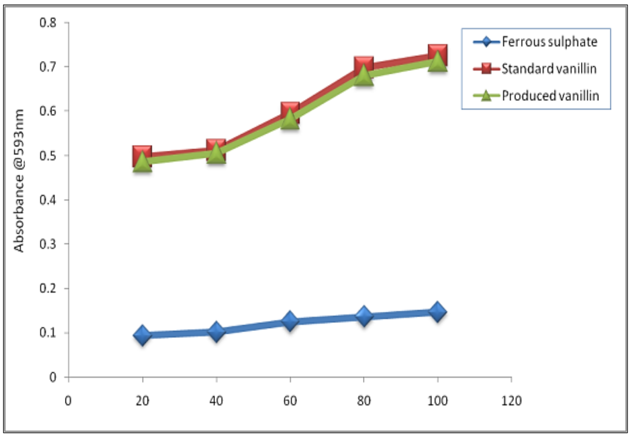


Fig 6 Absorbance vs. concentration curves for ascorbic acid, produced and standard vanillin in FRAP Assay

Thus, by comparing the absorbance verses concentration curves of standard and produced vanillin and their FRAP values; we can say that our produced vanillin depicts almost comparable ferric reducing ability to that of synthetic vanillin. So, we can say that vanillin produced by our method from oxidative depolymerisation of sawdust lignin using environment friendly ionic liquid [OMIM][OH] was found to have comparable antioxidant activity to that of standard vanillin by using FRAP assay.

CONCLUSION

This research work has summarized an environmentally benign efficient recyclable ionic liquid, [OMIM][OH] mediated production method for vanillin from the oxidative depolymerisation of lignin that was isolated from an easily available waste biomaterial of all kinds of wood working operations, sawdust or wood-powder. The antioxidant potential of our produced vanillin was evaluated using FRAP assay and it was observed that it displayed similar antioxidant potential to that of standard vanillin and thus can be used as an equally effective and valuable antioxidant agent or flavouring material like the high-quality pure vanillin that we use as a standard laboratory reagent.

Acknowledgement

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LITERATURE CITED

1. Ranadive AS. 1994. Vanilla-cultivation, curing, chemistry, technology and commercial products. *In*: (Eds) G. Charalambous, Spices, Herbs and Edible fungi. 1st Edition Elsevier, Science BV, Amsterdam. *Developments in Food Science* 34: 517-576.
2. Rao SR, Ravishankar GA. 2000. Vanilla flavor: Production by conventional and biotechnological routes. *Jr. Sci. Food Agriculture* 80: 289-304.
3. Sharma A, Verma SC, Saxena N, Chadda N, Singh NP, Sinha AK.2006. Microwave and ultrasound assisted extraction of vanillin and its quantification by high-performance liquid chromatography in *Vanilla planifolia. Jr. Sep. Sci.* 29: 613-619.
4. Borges Da Silva EA, Zabkova M, Araojo JD, Cateto CA, Barreiro MF, Belgacem MN, Rodrigues AE. 2009. An integrated process to produce vanillin and lignin-based polyurethanes from Kraft lignin. *Chem. Eng. Res. and Des.* 87: 1276-1292.
5. Sinha AK, Sharma UK, Sharma N. 2008. A comprehensive review on vanilla flavor: Extraction, isolation and quantification of vanillin and others constituents. *Int. Jr. Food Sci. Nutrition* 59(4): 299-326.
6. Fitzgerald DJ, Stratford M, Gasson MJ, Narbad A. 2005. Structure-function analysis of the vanillin molecule and its antifungal properties. *Jr. Agric. Food Chemistry* 53: 1769-1775.
7. Abraham DJ, Mehanna AS, Wireko FC, Whitney J, Thomas RP, Orringer EP. 1991. Vanillin, a potential agent for the treatment of sickle cell anemia. *Blood.* 77: 1334-1341.
8. Imanishi H, Sasaki YF, Matsumoto K, Watanabe M, Ohta T, Shirasu Y, Tutikawa K. 1990. Suppression of 6-TG-resistant mutations in V79 cells and recessive spot formations in mice by vanillin. *Mutant Research* 243: 151-158.
9. Burri J, Graf M, Lambelet P, Loliger J. 1989. Vanillin: more than a flavouring agent: a potent antioxidant. *Jr. Sci. Food Agriculture* 48: 49-56.

10. Mohamad Ibrahim MN, Sipaut CS, Mohamad Yusof NN. 2009. Purification of vanillin by a molecular imprinting polymer technique. *Sep. Purif. Technology* 66: 450-456.
11. Tarabanko VE, Fomova NA, Kuznetsov BN, Lavanchenko NM, Kudryashev AV. 1995. On the mechanism of vanillin formation in the catalytic oxidation of lignin with oxygen. *React. Kinet. Catal. L.* 55(1): 161-170.
12. Fargues C, Mathias A, Rodrigues A. 1996. Kinetics of vanillin production from kraft lignin oxidation. *Ind. Eng. Chem. Res.* 35: 28-36.
13. Shakeri A, Rad SA, Ghasemian A. 2013. Oxidative production of vanillin from industrial lignin using oxygen and nitrobenzene: A comparative study. *Intl. Jr. Farm and Alli. Science* 2(24): 1165-1171.
14. Hocking MB. 1997. Source lignin sulphite liquor Vanillin: Synthetic flavoring from spent sulfite liquor. *Jr. Chem. Educ.* 74(9): 1154-1159.
15. Shamsuri AA, Abdullah DK. 2010. Isolation and characterization of lignin from rubber wood in ionic liquid medium. *Mod. Appl. Science* 4(11): 19-27.
16. Fernandez Bolanos J, Felizon B, Heredia A, Guillen R, Jimenez A. 1999. Characterization of the lignin obtained by alkaline delignification and of the cellulose residue from steam-exploded olive stones. *Bioresource Technology* 68: 121-132.
17. Wallberg O, Jönsson AS. 2006. Separation of lignin in kraft cooking liquor from a continuous digester by ultrafiltration at temperatures above 100°C. *Desalination* 195(1): 187-200.
18. Hocking MB. 1997. Vanillin: Synthetic flavouring from spent sulfite liquor. *Jr. Chem. Educ*. 74: 1055-1059.
19. JI Botello, MA Gilarranz, F Rodriguez, M Oliet. 1999. Preliminary study on products distribution in alcohol pulping of *Eucalyptus globules*. *Jr. Chem. Technology and Biotechnology* 74: 14-148.
20. Barbera L, Pelach MA, Perez I, Puig J, Mutje P. 2011. Upgrading of hemp core for papermaking purposes by means of organosolvent process. *Ind. Crop Prod*. 34: 865-872.
21. Hallett JP, Welton T. 2011. Room-temperature ionic liquids: solvents for synthesis and catalysis. *Chem Rev.* 111: 3508-3576.
22. Yue C, Fang D, Liu L, Yi T. 2011. Synthesis and application of task-specific ionic liquids used as catalysts and/or solvents in organic unit reactions. *Jr. Mol. Liq.* 163: 99-121.
23. Shamsuri AA, Abdullah DK. 2010. Protonation and complexation approaches for production of protic eutectic ionic liquids. *Jr. Phys. Sci*. 21: 15-28.
24. Shamsuri AA, Abdullah DK. 2011. Synthesizing of ionic liquids from different chemical reactions. *Singapore Journal of Science and Research* 1: 246-775
25. Pu Y, Jiang N, Ragauskas AJ. 2007. Ionic liquid as a green solvent for lignin. *Jr. Wood Chem. Technology* 27: 23-33.
26. Xie H, Li S, Zhang S. 2005. Ionic liquids as novel solvents for the dissolution and blending of wool keratin fibers. *Green Chem.* 7: 606-608.
27. Shamsuri AA, Abdullah DK. 2012. A preliminary study of oxidation of lignin from rubber wood to vanillin in ionic liquid medium. *Oxid Commun*. 35(3): 767-775.
28. Pouteaua C, Dole P, Cathala B, Averous L, Boquillon N. 2003. Antioxidant properties of lignin in polypropylene. *Polym Degrad Stab.* 81: 9-18.
29. Jiebing L, Göran G, Kai T. 2009. Steam explosion lignins; their extraction, structure and potential as feedstock for biodiesel and chemicals. *Bioresour Technology* 100: 2556-2561.
30. Benzie IF, Strain JJ. 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymology* 299: 15-27.
31. Benzie IF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem.* 239(1): 70-76.
32. Oliveira CBS, Meurer YSR, Oliveira MG, Medeiros Q, Silva FON, Brito ACF, Pontes DL, Neto VFA. 2014. Comparative study on the antioxidant and anti-to xoplasma activities of vanillin and its resorcinarene derivative. *Molecules* 14: 5898-591.