Journal of Medicinal and Aromatic Plant Sciences 41(1-4), 2019; 50-57

Valorization of Cornmint (*Mentha arvensis*) distilled waste

DEEPAK KUMAR[1] • PRASHANT KUMAR[1] • PRAVEEN KUMAR SHARMA[1] • ASHWEEN DEEPAK NANNAWARE[1,2] • CHANDAN SINGH CHANOTIYA[1,2] •PRIYABRAT MOHAPATRA[3] • PRASANT KUMAR ROUT[1,2*]

Article History

Received: October 16th, 2019
Revised: November 30th, 2019
Accepted: December 4th, 2019

Key Words

Catalyst
Cellulose
Glucose
Isolation of bio-polymers
Levulinic acid
Mentha arvensis
Subcritical ethanol
Value addition

ABSTRACT

Natural l-menthol is a flavour and fragrance bearing compound, obtained from the essential oil of Cornmint or the mentholmint (Mentha arvensis). The valuable essential oil is obtained by hydrodistillation of green aerial part of the Metha Plant, leaving behind a distilled lignocellulosic biomass that is normally treated as waste. The volatile matter (73%), crystallinity and value addition (volatilization) of this biomass were estimated in this study. Cellulose (39%), hemicellulose (19%) and lignin (7%) were isolated using a laboratory fabricated 2 L double jacketed reactor. Isolated cellulose was enzymatically saccharified to glucose (610 mg/g) with 10 FPU/g loading of Cellic CTec2 (114 FPU/ml) at 50°C for 48 h. Further, glucose was utilized for the production of levulinic acid (LA) in subcritical-ethanol condition using Lanthanum (III) trifluoromethanesulfonate hydrate as a catalyst. At 150°C and 15 bar pressure, the glucose was converted to LA (54%) with purity of 96% in 90 min. In addition, the distillation condensate (~800 ppm) was also collected for recovery of water soluble essential oil through adsorption using Amberlite XAD-4 resin. The resin was desorbed in ethanol and after solvent removal the essential was isolated. The yield of recovered essential oil was 0.77 g /L, with menthol as major compound (82.3%).

INTRODUCTION

Strict environmental regulations have forced researchers and industry to find ways to convert lignocellulosic industrial biomass to transport fuel and other valuable biochemicals [11]. These cellulosic sources are being popularized due to their zero carbon credits, providing pollution free environment and potential to replace the fossil fuels. Cellulosic biomass is sustainable in nature and it is a potential candidate for deriving liquid bio-chemicals. India annually produces more than 7 million tons of Cornmint (*Mentha arvensis*) biomass and after hydro-distilled processing for essential oil production, that generates about 6 million tons of distilled biomass as waste. Menthol is one of the most important flavours and natural menthol production is more than 5000 tonnes per annum [4]. India fulfills 60% of World's menthol

[1]Chemical Sciences Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, Uttar Pradesh 226015, India
[2]Academy of Scientific and Innovative Research (AcSIR), New Delhi-110025, India
[3]Department of Chemistry, C.V. Raman College of Engineering, Bhubaneswar 752054, Odisha, India
*corresponding author, Email: pk.rout@cimap.res.in; prasantrout@gmail.com
Doi: https://doi.org/10.62029/jmaps.v41i2.Kumar

need by cultivating *Mentha arvensis* on an estimated land area of 1,62,800 hectare in the Northen states such as Uttar Pradesh, Bihar, Uttaranchal, Haryana and Punjab [4]. The essential oil, de-mentholized oil and menthol crystal are most used flavouring products of mentha oil. These are utilized as an essential ingredient in pharmaceutical, food and house-hold products [9]. Mint or menthol is one of the demanding flavours like other established favours such as chocolate, vanilla, citrus, etc. *M. arvensis* essential oil is isolated by hydrodistillation of freshly harvested or partially dried above ground biomass. After distillation, the spent waste biomass is generally used for burning purpose which is now being prohibited as per the new environmental guidelines. In the process of distillation, the biomass kept in contact with boiling water and hence automatically undergone a pre-treatment process that makes it an ideal choice for the production of valuable compounds. Isolation of cellulose from this distilled biomass is a vital step for getting the selective value added products.

Pre-treatment is an energy as well as cost intensive process in biorefinery. It generally disrupts the tissues in lignocellulosic biomass for the recovery of lignin, hemicellulose and cellulose. In the present study, a scale-up process is established for the selective segregation of biopolymers from this distilled biomass. Initially, this distilled biomass was characterized to know its chemical composition. The distilled biomass was first made free from easily extractable components and then subjected to the recovery of cellulose, hemicellulose and lignin. This, isolated cellulose was enzymatically hydrolysed for getting the glucose as per the optimised saccharification conditions developed in our laboratory[9].

There were a number of reports available for the synthesis of levulinic acid (LA) from cellulose or agriculture residues [11,12]. Biofine is operating the pilot facility for production of LA with capacity of 1 ton/day at South Glens Falls, USA. This is based on a mineral acid (H_2SO_4) assisted hydrolysis process, which is operated at 210-220^oC with a pressure of 25 bar. Crude LA (yield: 75%) is obtained after filtration of the powdery substance (char) [5]. Besides, there were a number of other process reported using heterogeneous catalysts [11,12]. Generally these reactions are carried out at near critical conditions of water. The critical temperature and pressure of water is 647 K and 220 bar, respectively. The limitations of these reported processes are as follows; the reaction was carried out in aqueous medium with 54% yield of LA was reported using the processed (ball milled cellulose) of pure grade microcrystalline cellulose. To overcome the water as solvent, whose critical conditions are very high and also it produced a number of undesired products, we have developed a process for synthesis of LA using subcritical (Sbc) ethanol as reaction medium. The critical temperature and pressure of ethanol is 243^oC and 64 bar, far below the critical conditions of water. The glucose, obtained by saccharification of isolated cellulose is used for the synthesis of LA in sub-critical ethanol using heterogeneous catalyst (Fig. 1a). LA can be treated as a model compound

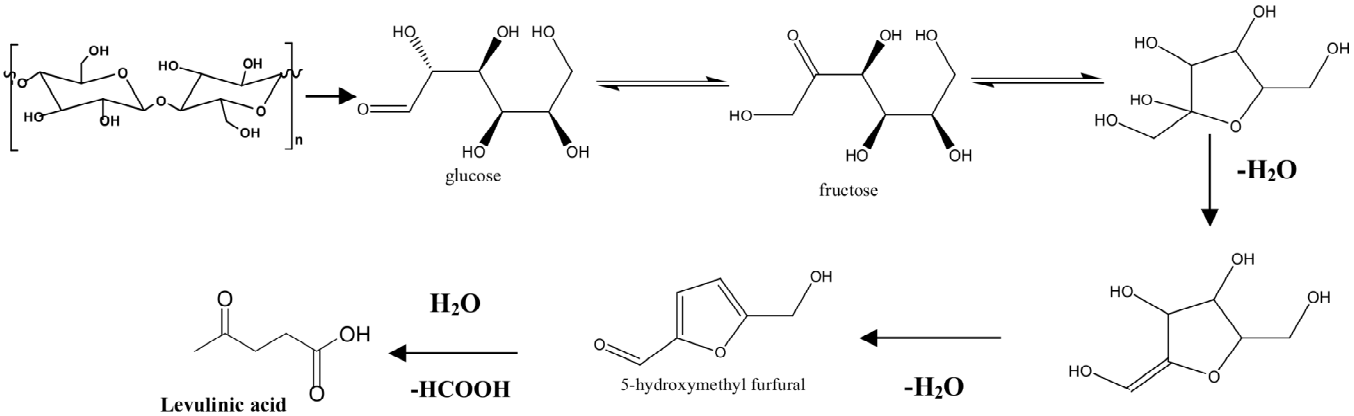

Figure 1a: Reaction steps for conversion of cellulose to levulinic acid

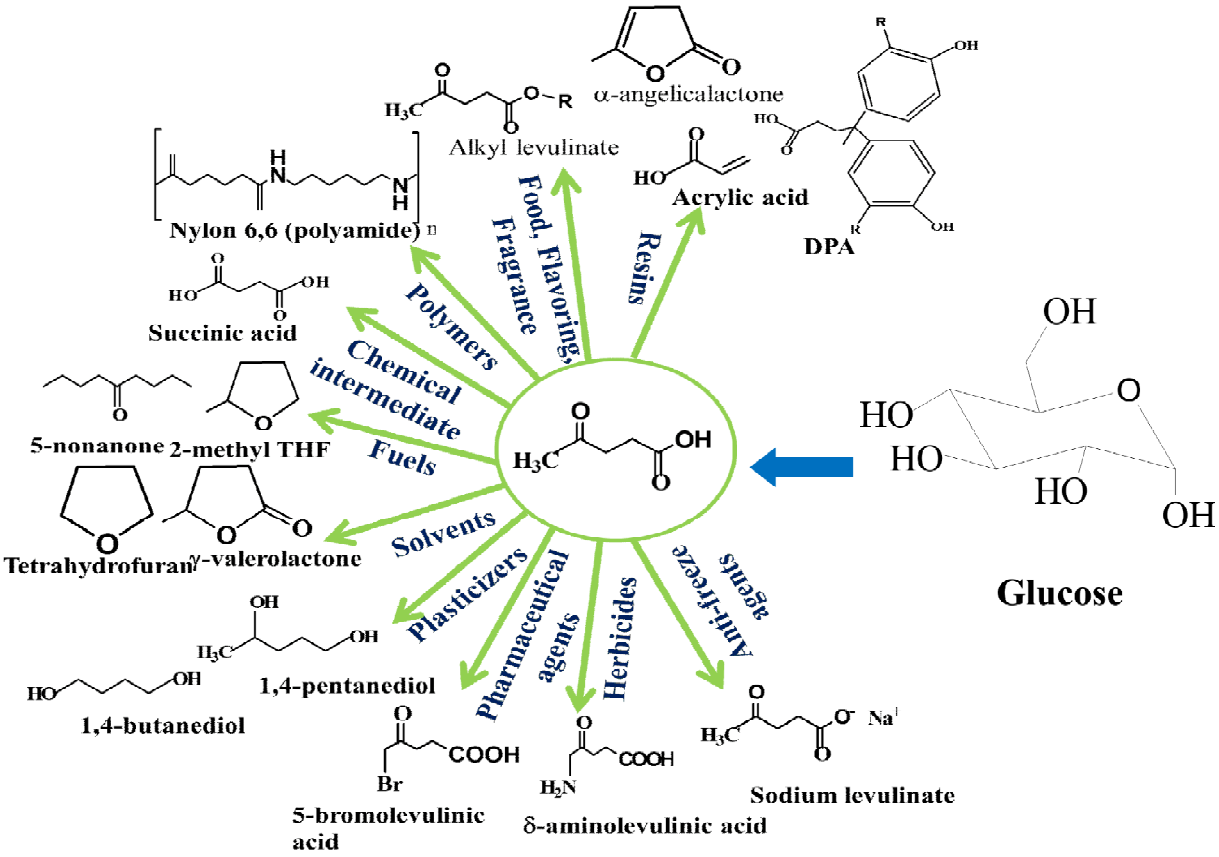

Figure 1b: Production of commodity products from levulinic acid

for manufacturing of several other useful commodity products (Fig. 1b). The economic value of some important LA derivatives are presented in Table 1 [3]. It has one ketonic functional group at C-4 position and acidic group at C-1 position, made it feasible to derive commercially important product such as MTHF (methyl-tetrahydrofuran), a compound of high calorific value clean fuel; d-aminolevulinic acid (DALA), a potential and biodegradable herbicide; succinic acid, a gasoline additive, etc. Similarly, the distillation condensate was collected for recovering of water soluble compounds to check the loss of essential oil at the time of distillation.

Table 1. Products from levulinic acid with its potential market demand in the year 2000*

Product	Use	Demand (million kg/ year)
MTHF	Fuel extender	4536-45359
DALA	Biodegradable herbicide	80-159
THF	Solvent	91
BDO	Monomer	91
Diphenolic acid	monomer	16

MTHF: methyl tetrahydrofuran, DALA: d-amino levulinic acid, THF: tetrahydrofuran, BDO: butanediol;*source:Bozell et al.(2000)

MATERIALS AND METHODS

Biomass, distillation condensate and chemicals

Distilled *Mentha arvensis* biomass and distillation condensate were collected from the CSIR-CIMAP field distillation unit. HPLC grade acetonitrile was procured from Merck, Mumbai. Standard carbohydrates, LA, microcrystalline cellulose, V_2O_5, TiO_2, Lanthanum(III) trifluoromethanesulfonatehydrate and Erbium(III)trifluromethanesulfonate were acquired from Sigma-Aldrich, Bengaluru. *Cellic CTec2* sample (100 mL) was kindly gifted by Novozymes, Bengaluru. Lignin and Amberlite XAD-4 were procured from TCI chemical, Hyderabad and s.d. fine chemicals, Mumbai, respectively.

Biomass characterization

Hydro-distilled biomass was dried under ambient conditions and then was macerated in a ball mill (0.5 mm). The volatile matter [2], and XRD analysis were carried out per our previous report [9]. TGA evaluation of distilled biomass and isolated biopolymer samples were analysed using Mettler-Toledo TGA/DSC 1 Star system. For this, 0.5 mg samples were taken in the silica crucible and run the analysis in the following temperature program of 30-500°C (5°C/min), 500-800°C (10°C/min) under nitrogen flow rate of 40 mL/min as purge gas. The acquired data were evaluated after taking the first derivative (DTG) for clear interpretation.

Scale-up process for the isolation of lignin, hemicellulose and cellulose

In one of our previous study, a separation protocol was established for the isolation of lignin, hemicellulose and cellulose from distilled *M. arvensis* biomass [9,13]. For scale-up study, two double jacketed 2 L capacity stirrer reactor have been designed and fabricated. Further isolation of the above bio-polymers was proceeded with the following steps: (i) biomass (250 g) was first treated with 1100 mL of methanol: hexane (1:1) in this reactor for 3 h to remove the waxy materials and chlorophyll. This de-waxed biomass was used for the isolation of lignin, hemicellulose and cellulose; (ii) For isolation of lignin, the above wax free biomass was contacted in 800 ml of 1% of $NaClO_2$ solution at 80°C for half an hour with periodical stirring. This $NaClO_2$ solution was filtered and residue was washed in 200 mL of distilled water. The filtrate was maintained to pH 2 using mineral acid for precipitating lignin that was then isolated through centrifugation; (iii) For isolation of hemicellulose, 700 mL of 5% NaOH solution was added to the delignified residue at 50°C for 30 min. Then the solution was filtered and the residue was washed with 150 mL of distilled water. The alkaline filtrate was maintained to pH 5 using the mineral acid and then 1000 mL of ethanol was appended in this solution for the precipitation of hemicellulose. Through filtration, the hemicellulose was separated and then it was washed with ethanol for removing any impurities; (iv) The remaining undissolved residue was treated with 200 ml of cooled 7% NaOH solution (0-4°C) and 200 ml of cooled 7% urea solution (0-4°C), concurrently for 30 min. Then, this solution was filtered and washed the residue with distilled water to get cellulose. This isolated cellulose was air dried for further using it in saccharification experiments as well as for characterization purpose.

Enzymatic saccharification for the production of glucose

Saccharification of the isolated cellulose was carried out using *Cellic CTec2* enzyme. The sccharification protocol and quantification of glucose were carried out as per our previous report [9].

Synthesis of levulinic acid from glucose

The synthesis of levulinic acid was carried out in a high pressure reactor of Autoclave Engineers make. Isolated cellulose (5 g) along with 50 mL ethanol was taken in the reactor. Then 2 ml of distilled water and 250 mg of catalyst was added to it. The reaction was carried out at 150 °C and 15 bar pressure in Sbc-ethanol for 90 min. After the reaction, the reaction mixture was filtered and concentrated. The LA samples were analysed by GC-FID (Flame Ionization Detector) and GC/MS analysis. The percentage composition of LA was determined in a Varian CP-3800 GC equipped with a capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) coated in Carbowax. FID was used as detector with temperature program: 40°C (1 min), 40-120°C (3°C/ min), 120°C (9 min), 120-140°C (2°C/min), 140°C (2 min), 140-250°C (5°C/ min), 250°C (5 min). The carrier gas was hydrogen at the flow rate of 1.7 mL/min. LA samples (0.06 mL) were injected through GC injection port. FID and injector were maintained at 310°C and 300°C, respectively. GC/MS was carried out in a PerkinElmer Auto System XL GC interface assembled with a Turbomass Quadrupole mass detector and samples were run using the above column. The injector, source and transfer line were maintained at 250°C. LA Samples (0.06 mL) were injected with a split ratio (1:45) and He was used as carrier gas with 10 psi pressure. The MS parameters as follows: EI 70 eV; mass range 40-450 amu.

Recovery of water soluble compounds from hydrosol

The recovery of water soluble compounds from distillation condensate was carried out using Amberlite XAD-4 resin (Fig. 2) as per our previous report [14]. For that, a column packed with 7 g of resin was used for slowly passing the 500 mL of distillation condensate. This process is carried out to collect water at the rate of 8 mL/min after passing through the resin bed. Then the trapped essential oil compounds were desorbed from the column using 100 mL of ethanol. Finally ethanol was removed in a rotary evaporator at reduced pressure and analyse the composition through GC-FID and GC/MS analysis.

Figure 2: Adsorptive recovery of water soluble essential oil from distillation condensate

RESULTS AND DISCUSSION

Characterization of distilled biomass

The distilled biomass was comprised of lipids and waxes (8-10%), lignin (16-18%), hemicellulose (28-30%) and cellulose (40-42%).

Crystallinity and devolatilization characteristics

Prominent X-ray diffraction peak was observed at 25° due to significant crystalinity arised from the cellulosic structure. Cellulose is a long chain structure of glucose molecule, which link one another through β-1,4-glycoside linkage to give brittleness of this natural polymer. In comparative XRD study, the biomass has more crystallinity to follow cellulose. On the other hand, lignin has least crystallinity due to irregular association of polyphenols, so broadly it is amorphous in nature. The significant crystallinity was observed in biomass due to cellulose structure, complex bonding among biopolymers (cellulose, hemicellulose and lignin) and also wax coating on the surface of the biomass [7,8]. Further, the distilled biomass had shown less crystallinity as compared to the fresh biomass [9]. Therefore the distilled biomass was favourable for enzymatic saccharification or chemical conversion due to its

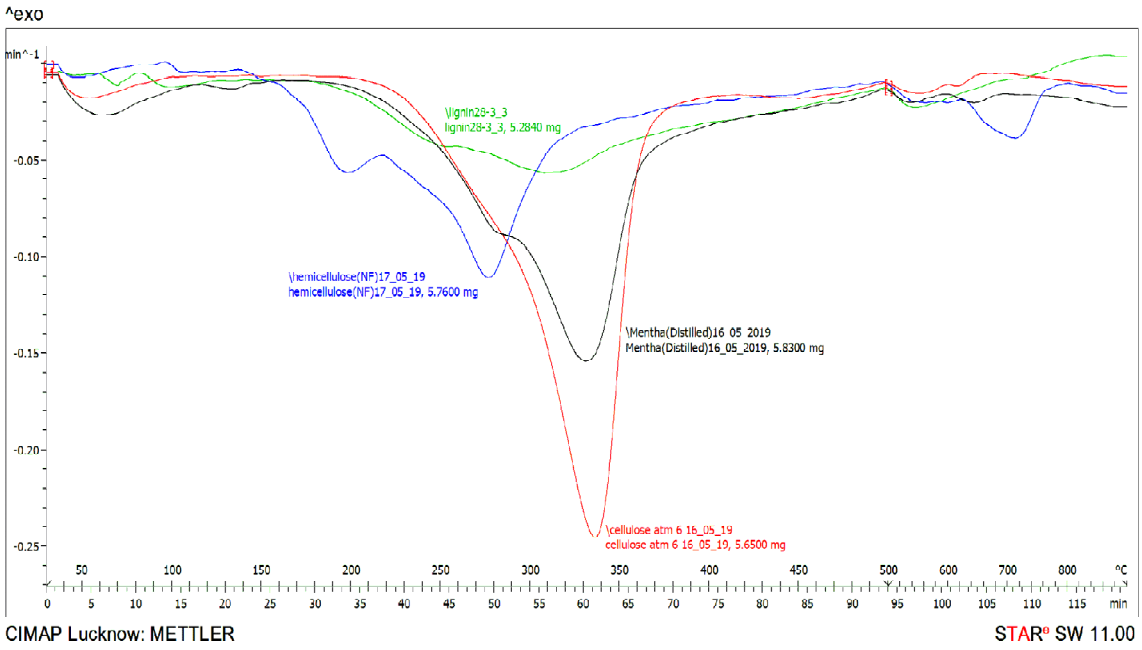

Figure 3: Comparative DTG analysis of distilled *M. arvensis* biomass with its isolated biopolymers

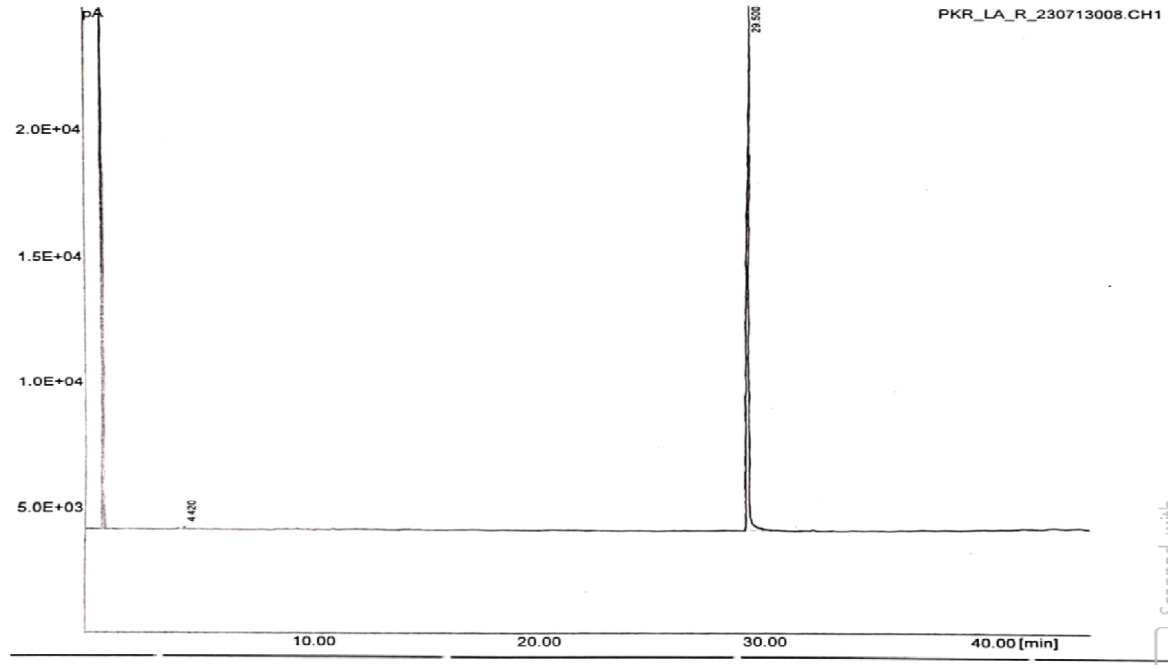

Figure 4: GC-FID of levulinic acid isolated from glucose reaction mixture

slight distorted assemblage among the biopolymers [9].

The estimated volatile fraction in the biomass was 73% [9]. The devolatilization pattern was better understood in TGA analysis and the data were suitably correlated to the percentage of lignin, hemicellulose and cellulose [10]. It was noticed that the loss of weight was 10% of the biomass at 200-250°C. The main mass loss was noticed at 250-350°C and then follow a regular pattern of continuous mass loss upto 800°C [9]. The mass losses at low temperatures were correlated to primary devolatization that predicts the loss of small volatile molecules including the moisture. At high temperatures, the mass losses were due to the degradation of long-chain molecules. Finally the data correlated that the mass loss at temperature less than 100°C was related to the volatiles, 110-130°C for vapours, 150-225°C for easily degradable components, 230-320°C for hemicellulose, 300-380°C for cellulose and 280-550°C for slow decomposition of lignin (Fig. 3).

Isolation of lignin, hemicellulose and cellulose

The advantage of the present process in comparison to those reported in the literature has been summarized in our previous report [9]. In the present scale-up isolation process, first the waxy materials (yield 8.5%) present on the surface of the biomass were removed using equal proportion of methanol and hexane. This organic solvent insoluble biomass was 88%. The isolated recovery of lignin and hemicellulose were 7% and 19%, respectively. The remaining insoluble portion, refers as crude cellulose was further treated with NaOH (7%) and urea (7%) solution for purifying of cellulose. The final recovery of the cellulose was 39%. The purity of these isolated bio-polymers was carried out as per the protocol outlined earlier[1].

Glucose production from isolated cellulose

It was estimated that the enzymatic activity of *Cellic CTec2* was 114 FPU/mL [9]. The glucose production (610 mg / g of cellulose) was attained in 48 h using *Cellic CTec2* enzyme. The cellulose was separated using alkali (NaOH) and further purified by simultaneous treatment with cold NaOH and urea solution. Combined treatment with later solution helped in increasing the surface area and porosity in the cellulosic structure. Therefore enzyme could easily access the active sites of cellulosic substrate for effective saccharification. Pre-treatment for removing a part of hemicellulose and lignin from the biomass and to follow the

saccharification had not significantly enhanced the glucose yield. Similar to our methodology and results, using *Cellic CTec* enzyme the production of glucose from pre-treated biomass was reported to yield 250 mg/g [15].

Green synthesis of levulinic acid (LA) from glucose

The weight of the extract was 2.7 g (54%). LA was identified through GC/MS and also co-elution using the standard. The mass spectra of LA were M^+: 116, M^+ - CH_3: 101, M^+ -CH_3-CO: 73. The GC-FID has shown that the percentage of LA in the extract was 95% (Fig. 4). For this reaction a number of catalysts have been tried for the synthesis of LA using V_2O_5, TiO_2, Erbium(III)trifluromethanesulfonate catalysts. These catalysts are produced good percentage of hydroxymethylfurfural, which is an intermediate in the synthesis of LA (Fig. 1b). But,use of these catalysts resulted in poor yield of LA. It is likely that, these catalysts could found difficulty in completing the last step of reaction from the intermediate hydroxymethylfurfual to LA (Fig. 1a). Whereas Lanthanum(III) triflurometh an esulfonatehydate contained H_2O molecule in the catalytic structure, which facilitated the hydrolysis reaction of hydroxymethylfurfural to LA.

Isolation and characterization of hydrosols

The amount of essential oil in distillation condensate was about 800 ppm. Therefore a significant percentage of essential oil is lost in the distillation water [6]. In the present process, the soluble essential oil was recovered by adsorption on XAD-4 resin (0.77 g /L). The essential oil was recovered by desorption using ethanol and this resin was re-generated for further use. It was noticed that nearly 770 ppm of soluble compounds were recovered from the distillation condensate. It contained menthone (1.2%), isomenthone (1.7%), menthol (82.4%) and menthyl acetate (1.5%), so that the valuable oxygenated terpenoids are recovered. Therefore this is an environment-friendly and cost-effective process for recovering the essential oil lost during the distillation process and goes into condensate. Further qualitative analysis of this recovered essential oil is in progress.

CONCLUSION

India generates huge amounts of distilled *M. arvensis* biomass (6 million tons). After recovery of high value essential oil through hydrodistillation, the remaining hot-water treated biomass can be used for the production of glucose or synthesis of LA. The present study comprised of biomass composition analysis, isolation and characterization of biopolymers, saccharification of isolated cellulose and finally green synthesis protocol of LA from glucose. In broad sense, conversion of distilled biomass to LA can help to mitigate the waste disposal problems. Presently, the scale-up isolation of cellulose (39%), hemicellulose (19%) and lignin (7%) were carried out using 2 L glass reactor. The crystallinity of isolated cellulose was significantly reduced as compared to the crude biomass, which was a positive impact for efficient production of glucose and LA. The distilled biomass contained good amounts (73%) of volatile materials. TGA study further validated that more than 80% of biomass was volatile in nature. The biomass characterisation facilitates the use of the biomass for conversion to bio-gas, bio-oil, bio-ethanol, and also catalytic pyrolysis for the production of phenolics (valuable aroma molecules). Isolated cellulose was enzymatically saccharified to glucose (610 mg/g) with 10 FPU/g loading of *Cellic CTec2* (114 FPU/mL) at 50^{o}C for 48 h.

The catalytic process for synthesis of LA from glucose using Sub-ethanol is reported first time here with 54% of yield. LA is a potential starting precursor molecule to derive a variety of commodity products due to its unique structure and also derived a number of furan chemicals for the fragrance industry. Amberlite XAD-4 is an effective polymeric resin for complete recovery of essential oil from distillation condensate. Further, proper integration of additional XAD-4 column with the distillation unit is able to check the loss of essential oil in distillation condensate.

ACKNOWLEDGEMENTS

Authors are thankful to SERB, Department of Science & Technology, India (EMR/2016/005359) for fellowship to Prashant Kumar and, Department of Biotechnology, India (BT/PR9694/PBD/26/402/2014) for financial support to carry out this work. We are grateful to Director, CSIR-CIMAP, Lucknow for providing the necessary laboratory facilities and other logistic support.

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