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Evaluation on Efficiency of Pyroligneous Acid from Palm Kernel Shell as Antifungal and Solid Pineapple Biomass as Antibacterial and Plant Growth Promoter

(Penilaian terhadap Keberkesanan Asid Piroligneus daripada Tempurung Isirung Sawit sebagai Antikulat dan Sisa Pepejal Nanas sebagai Antibakteria dan Promoter Pertumbuhan Tumbuhan)

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ABSTRACT

Generation of huge volumes of lignocellulosic biomass from agricultural sector is of concern due to its direct effects on the depletion of overall environmental quality. Conversion of biomass into solid biofuel through pyrolysis reaction has become one of the solutions to manage the abundance of biomass. Pyroligneous acid (PA) produced from the condensation of smoke generated during biomass carbonization process has the potential to be applied in various applications based on the diverse active chemical compounds present. In this study, PA obtained from palm kernel shell (PKS) was evaluated for antifungal activity and solid pineapple biomass (PB) was evaluated for antibacterial and plant growth promoter activities. Higher antifungal activity was determined for crude PA from PKS (PA-PKS) and dichloromethane-extract (DPA-PKS) with 0% coverage area when evaluated using rubber wood blocks against mold and blue sapstain after for 4 weeks of observation. This antifungal activity can be attributed to the presence of phenols and its major derivatives as suggested from the GC-MS and FTIR analysis. Concentrated PA from PB displayed good antibacterial capabilities with almost similar growth inhibition for Escherichia coli *(13±1 to 20*±1 *mm) and* Corynebacterium agropyri *(20±1 mm). PA-PB also showed good potential as PGP where the addition of 2% (v/v) of PA-PB into the fertilizer for okra plant resulted in highest number of leaves and fruits while 4% (v/v) PA-PB managed to give highest plant height, longest root, heaviest fruits and biggest leaf diameter. Thus, this study successfully demonstrated the potential use of PA obtained from lignocelluosic biomass in various applications.*

Keywords: Antibacterial; antifungal; plant growth promoter; pyroligneous acid

ABSTRAK

Lambakan biojisim lignoselulosa berpunca daripada aktiviti sektor pertanian adalah membimbangkan disebabkan oleh kesan langsungnya terhadap pengurangan kualiti alam sekitar. Penukaran sisa biojisim kepada bahan bakar biopepejal melalui pelbagai proses seperti pirolisis adalah salah satu langkah untuk menyelesaikan masalah lambakan biojisim. Asid piroligneus (PA) yang terhasil daripada proses penyulingan asap semasa proses pengkarbonan biojisim mempunyai potensi untuk digunakan dalam pelbagai aplikasi berdasarkan kehadiran pelbagai sebatian aktif kimia. Dalam kajian ini, PA daripada tempurung isirong sawit (PKS) dan biojisim nanas (PB) telah dinilai untuk aktiviti anti-kulat, anti-bakteria dan penggalak pertumbuhan tumbuhan (PGP). Aktiviti anti-kulat tertinggi ditunjukkan oleh PA-PKS mentah (PA-PKS) dan ekstrak diklorometana PA-PKS (DPA-PKS) dengan 0% luas litupan permukaan oleh kulapuk dan sapstain biru pada blok kayu getah selepas pemerhatian selama 4 minggu. Aktiviti antikulat adalah disebabkan kehadiran fenol dan terbitan utamanya berdasarkan analisis GC-MS dan FT-IR. PA pekat daripada PB menunjukkan keupayaan anti-bakteria dengan tingkat perencatan pertumbuhan yang sama untuk Escherichia coli *(13±1 to 20*±1 *mm) dan* Corynebacterium agropyri *(20±1 mm). PA-PB juga menunjukkan potensi untuk digunakan sebagai PGP dengan penambahan 2% (v/v) PA-PB kepada baja untuk pokok bendi memberikan jumlah daun dan buah tertinggi manakala 4% (v/v) PA-PB memberikan pertumbuhan pokok tertinggi, akar terpanjang, buah terberat dan diameter daun terbesar. Kesimpulannya, kajian ini telah berjaya menunjukkan potensi PA daripada biojisim lignoselulosa dalam pelbagai aplikasi.*

Kata kunci: Antibakteria; antikulat; asid piroligneus; promoter pertumbuhan tumbuhan

INTRODUCTION

Agricultural sector is one of the biggest economic contributors for nearly all countries in the world. This scenario has resulted in the generation of huge volumes of wastes including solid lignocellulosic biomass. Conversion of lignocellulosic residual plant biomass into various high value products including biofuels, organic chemicals, enzymes, enhanced animals feeds and

energy (Howard et al. 2004) has become an alternative to overcome the environmental problems resulting from its disposal. Of these, conversion of biomass into charcoal from carbonization process through pyrolysis reaction has been widely applied. Pyroligneous acid (PA) is an acidic aqueous by-product which was obtained from the distillation of smoke generated during carbonization process. It comprises of water (80-90%) and a mixture of complex organic compounds (10-20%) such as phenolics, ketones, furan, pyran and organic acids. PA is known for its use in odour removal, smoke flavouring, able to promote plant growth, act as herbicides and pesticides, inhibiting bacterial growth, antioxidant and anti-inflammatory activity (Ho et al. 2013; Lee et al. 2011a, 2011b, 2010; Loo et al. 2008; Nakai et al. 2007; Rungruang & Junyapoon 2010).

There are numerous types of lignocellulosic biomass that has been utilized for production of PA. Bamboo has been one of the popular sources of PA especially in Asian region due to its widespread population (Ho et al. 2013; Kimura et al. 2002; Lee et al. 2011b; Marumoto et al. 2012; Wu et al. 2015). PA production has also been reported from various woody biomasses such as oak wood (Lee et al. 2011a), walnut branches (Wei et al. 2010a), mangroves (Ibrahim et al. 2013; Lee et al. 2011b; Loo et al. 2008), rubberwood (Lee et al. 2010; Ratanapisit et al. 2009), mixed hardwood sawdust, oil palm trunk (Lee et al. 2010), Eucalyptus wood (Rungruang & Junyapoon 2010; Souza et al. 2012), Chinese fir (Wu et al. 205) and durio bark (Oramahi & Diba 2013). Agricultural waste materials such as walnut shell (Ma et al. 2011; Wei et al. 2010b), cotton stalk (Wu et al. 2015), palm kernel cake, cassava pulp residue (Weerachanchai et al. 2011) and pineapple (Mathew et al. 2015) also have been used for production of PA. Other source of PA is herbaceous biomass namely rosemary leaves (Ma et al. 2013) and *Schisandra chinensis* (Ma et al. 2014). The PA produced from these biomasses has been studied for its bioactivities and possible application has been extensively explored. Nevertheless, there is still limited study available on the bioactivities of PA obtained from palm kernel shell (PKS) and pineapple biomass (PB). The objective of this study was to investigate the potential bioactivities of PA obtained from PKS as antifungal and PB as antibacterial and plant growth promoter.

Materials and Methods

PA from palm kernel shell of oil palm (Tenera variety) pyrolyzed at 350°C and PA from pineapple solid biomass of pineapple plant (Josapine hybrid variety) pyrolyzed at 600°C used in this study was provided by the Malaysian Palm Oil Industry Board (MPOB) Station in Bangi, Selangor and Maju Jaya Organik Sdn. Bhd., Kluang, Johor, respectively. Crude PA from palm kernel shell (PA-PKS) was extracted using liquid-liquid extraction method; meanwhile the crude PA from pineapple solid biomass (PA-PB) was only concentrated to remove the water. Crude PA-PKS was first extracted using dichloromethane (CH_2Cl_2, 84.93 g/mol, 99.5%, QRec) with a ratio of 1 CH_2Cl_2:1 PA-PKS. The organic phase (bottom layer) was collected and termed as DPA-PKS while the upper layer (aqueous phase) was re-extracted twice using fresh dichloromethane. Both organic phases (bottom layer) were collected after every extraction. The upper layer (aqueous phase) was also collected after extracted and termed as APA-PKS. Both fractions collected were concentrated using rotary vacuum evaporator (Heidolph, Germany) at 50°C and 90 mbar/torr until the volume is 1/10 of its original volume. The crude PA from pineapple solid biomass (PA-PB) was concentrated using rotary vacuum evaporator (Heidolph, Germany) at 78°C. It was then further dried in a vacuum desiccator until the volume is 1/60 of its original volume to obtain concentrated PA-PB and termed as CPA-PB. The PA-PB was also further extracted according to method described for PA-PKS using dichloromethane for further characterization of chemical compounds. The dichloromethane extract PA-PB was termed as DPA-PB.

Antifungal Activity of Pyroligneous Acid

The fungi resistance test (antifungal activity) was carried out according to ASTM D4445-10: Standard Test Method for Fungicides for Controlling Sapstain and Mold on Unseasoned Lumber (Laboratory Method) involving *Aspergillus niger* and *Botryodiplodia theobromae* at Wood Mycology Laboratory, Forest Research Institute Malaysia (FRIM), Selangor. Both fungi were cultured on malt extract agar and incubated at 27°C in the growth chamber with relative humidity of 70% until the agar is fully covered. After 3 weeks, 120 pieces of rubberwood (*Hevea brasiliensis*) blocks (length - 70 mm, width - 20 mm, thickness - 7 mm) were placed onto the growth fungi culture for molds and blue sapstain test. Untreated wood block was used as control and 20 pieces of wood blocks were used for each treatment including control. All the test specimens were impregnated in five types of treatment including RPA-PKS, DPA-PKS, APA-PKS, dichloromethane (DCM) and water at 90 psi for 1 h and left to submerge in the treatment for another 3 h at room temperature. Then, the wood blocks were taken out and the excess solution was wiped off and allowed to dry to constant weight for 3 weeks at room temperature. The rubberwood blocks were exposed to mold and sapstain in a sealed petri dish at 27°C and 70% RH. The test was carried out for a period of 4 weeks with standard weekly observation by calculating the number of days for mold to start growing on the test block together with coverage area of mold on the test block.

Antibacterial Properties of Pyroligneous Acid

Two types of Gram negative bacteria namely *E. coli* and *C. agropyri* were used in this study. Antimicrobial susceptibility assay was carried out using the Kirby-Bauer

antibiotic testing method (Bauer et al. 1966) as follows; 10 µL of RPA-PB and CPA-PB were placed on pre-sterilized 10 mm filter-paper discs. The test plates were then incubated at 37°C for 24 h for *E. coli* and at 26°C for 24 h for *C. agropyri*. The results of the average diameter (in mm) of the inhibition zone surrounding the discs containing the test solution were recorded.

PYROLIGNEOUS ACID AS PLANT GROWTH PROMOTER

Okra seeds were used as plant materials in this study to evaluate the potential of pyroligneous acid as plant growth promoter. The seedling soil was obtained from Kastura Garden Nursery, Skudai, Johor Bahru, while base soil was provided by Agricultural Unit, Department of Technical & Vocational Education, Faculty of Education, Universiti Teknologi Malaysia (UTM). The base soil consists of organic soil, red soil and sand (70:20:10) with pH of 5.64. Commercial organic fertilizer was provided by the Institute of Bioproduct Development, UTM. The seedling medium was prepared by mixing the seedling and base soils at a ratio of 2:1. It was then transferred into the germination pot until 3/4 of the pot's volume was filled, followed by slow watering until constant water flow was observed through the holes at the bottom of the seedling pot. After left for 1 h, 2-3 okra seeds were sown on the wet seedling medium. All germination pots were watered twice daily until the plantlet grown to 6-10 cm height with 3 leaves. The plantlets were then transferred into polybag (half-filled) containing mixture of base soil and organic fertilizer (one tea-spoon). The soil moisture content was maintained by watering twice daily. The effect of PA-PB as potential plant growth promoting agent for okra plant was evaluated as follows; 20 mL of 2.0-10.0% v/v of PA-PB solutions (termed as PA1 - PA5) were applied onto respective seedling mediums. Three plants were used for each concentration. Growth of the plant was monitored for 12 weeks where weekly monitoring of height, number of leaves, diameter of leaves, number of fruits produced, weight of fruits and general plant condition, were carried out.

QUALITATIVE AND QUANTITATIVE ANALYSIS

The functional groups present in all of the PA fractions used in this study were analyzed using FT-IR spectroscopy (Perkin Elmer, Waltham, MA, USA). The PA sample was dripped on thin films between the KBr plate and the spectrum was recorded in the wave number ranged from 500-4000 cm^{-1}. One particular fraction namely CPA-PB was determined for acetic acid using the following procedure; acetic acid were analyzed using waters HPLC system (Milford, MA, USA) consisting a pump and system controller (Model 2695) and photo-diode array detector (Model 966). These compounds separation was done by a reversed phase column (4.6 × 250 mm, 5 µm) (Phenomemex, Torrance, CA, USA). The separation was achieved by flow rate of 1 mL/min with 1 mmol/L H_2SO_4 + 8 mmol/L Na_2SO_4 (pH2.8) and samples (10 µL) were introduced into the column using an autosampler. The detection was monitored at 210 nm and data were integrated by Empower 2 software (Waters) (Milford, MA, USA). Another fraction namely DPA-PKS and DPA-PB were analyzed using GC-MS (Perkin Elmer, Waltham, MA, USA) as follows; 1 mL of DPA-PKS or DPA-PB was diluted with 20 mL of methanol. The diluted sample was injected into the capillary column with flow rate of 1.2 mL/min using helium as carrier gas. The column temperature was held at 50°C to 325°C and the run time was 42.5 min. Compounds were identified by comparing with the National Institute of Standards and Technology (NIST) library.

STATISTICAL ANALYSIS

The results obtained from the plant growth promoter study were analysed using SPSS16 software and was expressed in mean ± standard deviation (S.D). The data were subjected to analysis of variance (ANOVA) using Duncan's multiple range test with 5% significant.

RESULTS AND DISCUSSION

ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF PYROLIGNEOUS ACID

A. niger mold started to grow on the wood treated with APA-PKS (0.6%), dichloromethane (DCM) (0.3%), water (47.8%) and untreated wood blocks (5%) at week 1 meanwhile *B. theobromae* sapstain started to grow on the wood treated with APA-PKS (1.8%), water (22.45%) and untreated wood blocks (7.35%) at week 1. Wood blocks treated with PA-PKS and DPA-PKS extracts show highest inhibition against *A. niger* mold and *B. theobromae* sapstain with 0% coverage area while application of APA-PKS resulted in the lowest inhibitory profiles for *A. niger* mold and *B. theobromae* sapstain with more than 75% coverage area after 4 weeks observation. For the control sets, formation of colonies for *A. niger* mold and *B. theobromae* sapstain was observed for both water treated wood blocks and untreated wood blocks since week 1 of treatment. This was due to the increase in water activity that enhanced the growth of mold and sapstain. Figure 1(a) and 1(b) shows the percentage of fungi formation on the surface of wood blocks treated using various concentrations of PA from week 1 until week 4.

For the antibacterial test, clear inhibition zones were observed around the filter paper discs impregnated by CPA-PB compared to PA-PB and control which shows zero zone of inhibition against *E. coli* and *C. agropyri* (Figure 2). The CPA-PB fraction showed good antibacterial properties with inhibition zone diameters of 20±1 to 13±1 mm for *E.coli* and 20±1 mm for *C. agropyri* (Table 1). This is indeed an interesting finding as CPA-PB may have the potential to apply as antibacterial agent for both the Gram negative and Gram positive bacteria, respectively.

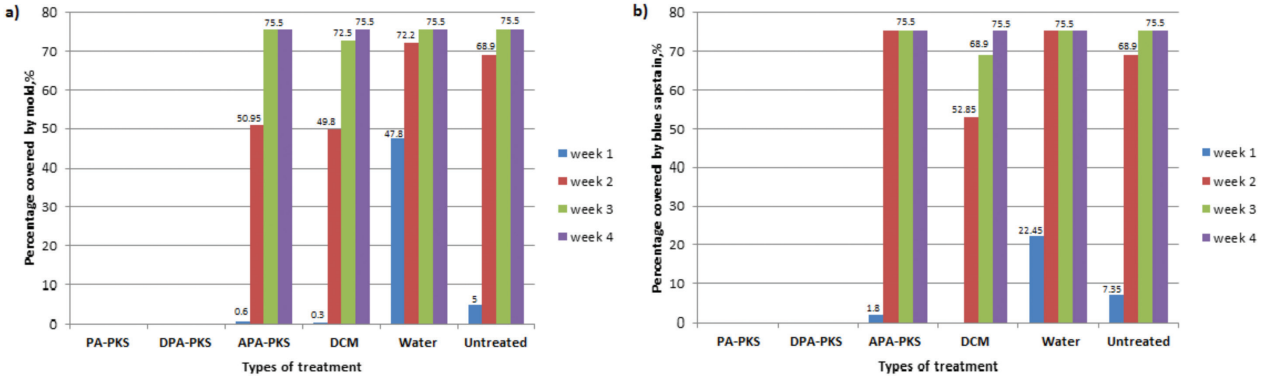

FIGURE 1. Percentage of fungi covering the wood blocks (a) mold and (b) blue sapstain for week 1 until week 4

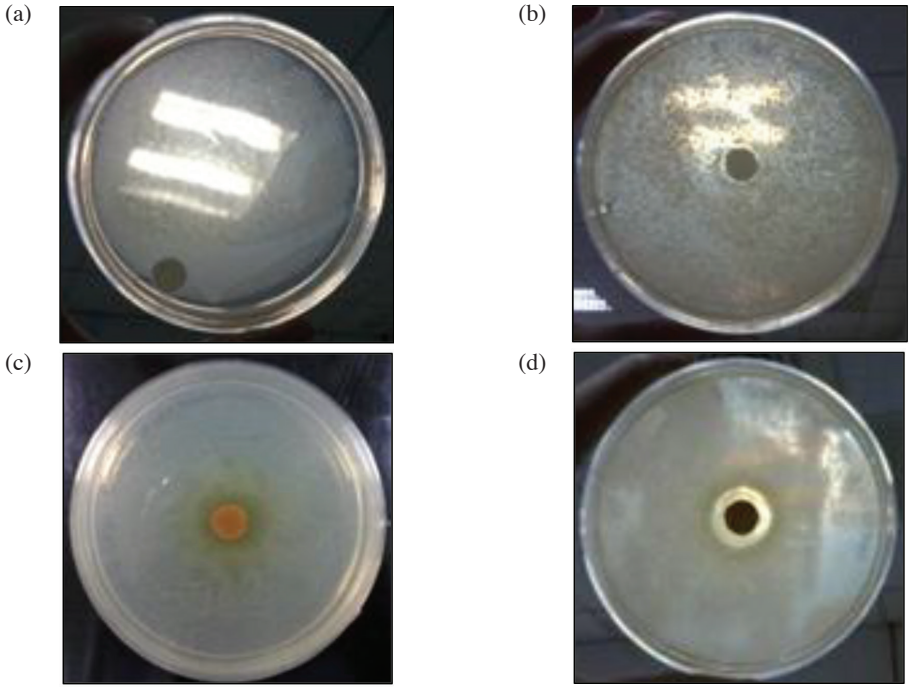

FIGURE 2. Antibacterial activity of PA-PB and CPA-PB based on inhibition zone (a) zero inhibition of *E. coli*, (b) zero inhibition of *C. agropyri*, (c) positive inhibition of *E. coli* and (d) positive inhibition of *C. agropyri*

TABLE 1. The antibacterial activity of PA-PB and CPA-PB fractions

Bacteria	Diameter of inhibition zone (mm)		
	Control	PA-PB	CPA-PB
Escherichia coli	Negative	Negative	13±1 to 20±1
Corynebacterium agropyri	Negative	Negative	20±1

PYROLIGNEOUS ACID AS PLANT GROWTH PROMOTER

There were no significant differences observed on heights and leaves diameter for the okra plant when different concentrations of PA-PB were applied onto soil mixture and fertilizer (Table 2). Addition of PA2 gave the highest plant height (105.87±32.61 cm) and leaves diameter (25.00±2.29 cm). PA2 also resulted in the highest fruit weight (34.11±11.41 g) and root length (51.73±7.17 cm) while PA1 gave the highest leaves number (19.00) and fruit number (6.00). Quite significant difference was observed for fruit's weight and fruit's number for PA1 and PA2 compared to PA5 and control. However, the addition of PA3 and PA4 did not result in any significant difference for all parameters evaluated related to the rest of the PA fractions. The root length shows a difference between PA1, PA2 and PA3 compared to PA5 while PA4 and control sample

TABLE 2. Result of okra plant growth with different treatment of PA-PB

Treatments	pH	PH, cm	LD, cm	FW, g	RL, cm	LN	FN
PA1	5.21	100.30±9.56^{a}	24.70±1.99^{a}	29.37±9.75^{b}	42.50±5.07^{b}	19.00^{b}	6.00^{b}
PA2	4.03	105.87±32.61^{a}	25.00±2.29^{a}	34.11±11.41^{b}	51.73±7.17^{b}	16.00^{b}	5.00^{b}
PA3	3.80	88.97±23.42^{a}	23.50±3.12^{a}	18.58±0.68^{a,b}	40.97±15.84^{b}	11.00^{a,b}	4.00^{a,b}
PA4	3.76	87.47±32.83^{a}	19.27±6.21^{a}	15.76±13.42^{a,b}	35.50±12.67^{a,b}	15.00^{a,b}	4.00^{a,b}
PA5	3.66	63.67±18.58^{a}	15.00±11.53^{a}	7.24±12.54^{a}	17.80±5.09^{a}	13.00^{a,b}	1.00^{a}
Control	-	78.70±12.87^{a}	20.00±9.90^{a}	6.05±10.47^{a}	33.70±13.15^{a,b}	7.00^{a}	1.00^{a}

*PH = plant height; LD = leaves diameter; FW = fruit weight; RL = root length; LN = leaves number; FN = fruits number; different letter indicate the significant different among the treatment (p>0.05)

showed no difference with all other treatments. Treatment using PA1 and PA2 for leaves number shows no significant different with treatment using PA3, PA4 and PA5, but shows significant different with control.

An increase of PA-PB concentration from 2.0 to 4.0% shows improvement on plant growth. According to Ishii et al. (1990), application of relatively low pyroligneous acid concentration may stimulate seedling growth, thus increases the germination rate of vegetable seed. The use of higher PA-PB concentrations (6-10%) resulted in decreased plant growth which may be attributed to the increase in soil acidity which is unfavourable for plant growth due to its direct effect on roots's growth (Foy 1992). Root is a crucial part of plant that supplies nutrient and water from soil to all other part of plant with the longest root generally gave excellent nutrient absorption. The diameter of leaves is also important to enhance the photosynthesis process by providing wide surface area for the reaction to occur.

FTIR, HPLC AND GC-MS CHARACTERIZATION

For pyroligneous acid from palm kernel shell sample, the broad band of hydroxyl stretching group between 3500-3200 cm^{-1} indicates the presence of alcohols, water and phenols (Figure 3). This peak is weak for DPA-PKS extract showing that aquaphilic compounds diffused into the aqueous phase namely APA-PKS extract. The C=O stretching between 1750-1650 cm^{-1} represent the ketones, aldehydes and carboxylic acids groups. The sharp peak shown in the range of 1500-1650 cm^{-1} from DPA-PKS extract spectrum indicated the presence of alkenes. The peaks in the range of 1300-950 cm^{-1} attributes to the presence of different alcohols, phenols, ethers and esters. The APA-PKS extract shows the weaker peak indicates that aromatic and non-polar organic of the compound presences were diffused to the organic phase DPA-PKS extract. The FT-IR analysis showed the probable compounds such as ketone, aldehyde, phenols and carboxylic acid play an importance role in antifungal activity.

The potential chemical compounds presence in pineapple pyroligneous acid based on the functional group also was analyzed using FT-IR spectroscopy. The FT-IR spectrum showed the presence of hydroxyl and carbonyl groups with prominent peaks at 3442 and 1634 cm^{-1}, respectively (Figure 4). Meanwhile, the absorption at 1416 cm^{-1} can be attributed to the C-H stretching from methylene group, C-O ether and aromatic compounds were also indicated by the absorption at 1062 and 695 cm^{-1}, respectively (Figure 4). The decomposition of cellulose and hemicellulose in pyrolysis process produced carbonyl groups such as carboxylic acid, aldehydes and ketones, together with phenol and alcohol. The C=O stretching group was observed at frequency 1634 cm^{-1}. Based on the result, pineapple pyroligneous acids were acidic as the FT-IR results showed the presence oxygenated functional groups of O-H, C=O, C-O and other aromatic compounds. According to Baimark and Niamsa (2009), organic acid and phenolic compounds of pyroligneous acid have been reported to be a main role in the antimicrobial activity. Table 3 shows the potential chemical compounds presence in the pyroligneous acid of palm kernel shell and pineapple biomass as well as other types of biomass based on the functional group analyzed using FT-IR spectroscopy.

Other FTIR analysis using different types of PA also reported similar profiles for chemical constituents present. PA from mangrove, bamboo (Lee et al. 2011b), rice straw, jute-stick and bagasse (Islam et al. 2012) recorded the stretching vibration between 3600-3200 cm^{-1} which indicated the presence of water impurities and other polymeric alcohols in the pyrolytic oil. Meanwhile, the absorbance peak between 1775-1650 cm^{-1} represent the C=O stretching vibration, displaying the existence of ketones, aldehydes and carboxylic acids (Islam et al. 2012). The O-H vibration is corresponding to water vapour peak, where the hydroxyl group easily forming hydrogen bond within or between molecules fell off during pyrolysis of biomass. This resulted in the formation of water and carbonyl groups. The pronounced oxygenated functional groups of O–H; C=O; C–O and aromatic compounds showed that the PAs were highly oxygenated and therefore, very acidic, as have also been indicated by the elemental composition and the pH value. The presence of hydrocarbon groups C–H; C=C and alcohols indicate that the liquids have the potential to be used as fuel (Islam et al. 2012).

HPLC analysis of CPA-PB showed that acetic acid as main component with concentration of 22.121 g/L

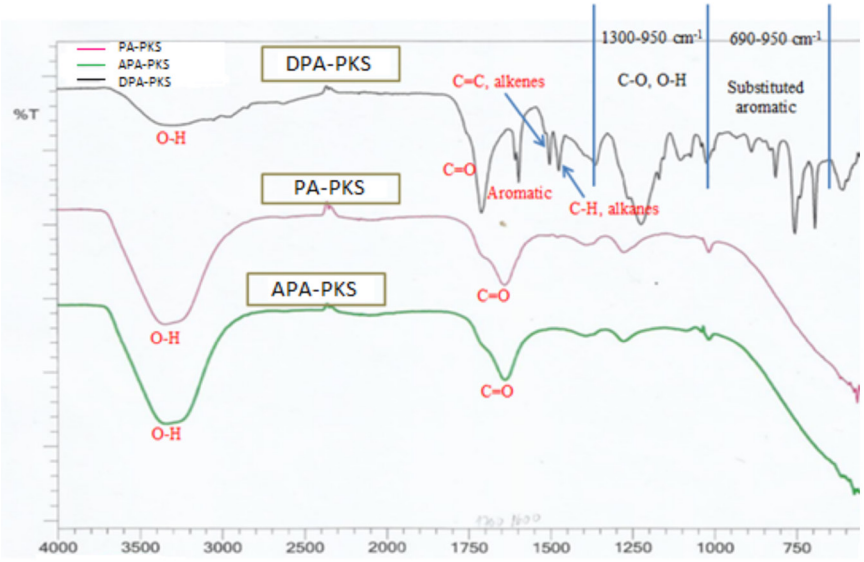

FIGURE 3. FTIR spectrum of DPA-PKS, PA-PKS and APA-PKS

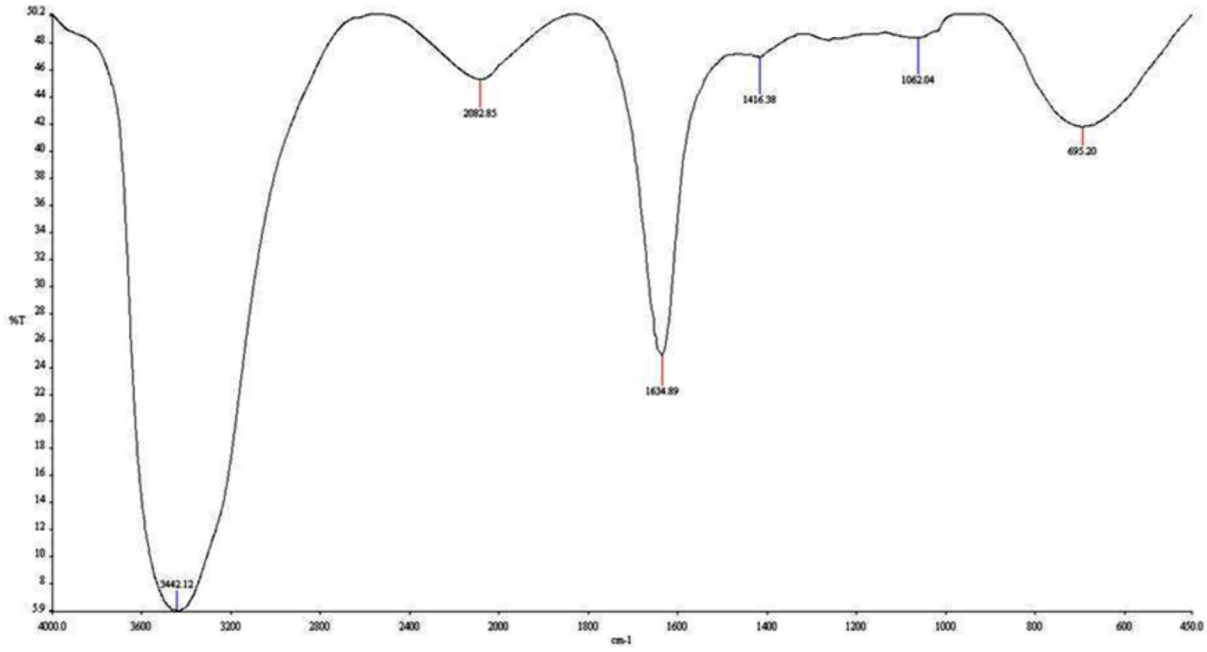

FIGURE 4. FTIR spectrum of CPA-PB

compared to PA-PB with concentration of 16.764 g/L (UTM-MPOB report) (Figure 5). Inhibition of *E. coli* investigated by Adams and Hall (2007) showed that acetic acid possess antibacterial activity. Thorp et al. (2007) also obtained positive result of inhibition zone for Gram positive and Gram negative using acetic acid. Thus, CPA-PB exhibit greater antimicrobial activity compared to PA-PB due to its high acetic acid concentration.

Chemical characterization by GC-MS has identified 96 compounds in the DPA-PKS extract (Table 4) with the highest compound being phenols and its derivatives group, accounting for 76.42%. The major compounds of phenols and its derivatives group were phenols (49.05%), 2-methoxyphenol (5.60%), 4-methyl-2-methoxyphenol (2.70%) and 4-ethyl-2-methoxyphenol (1.58%). Following this are the furan and pyran derivatives (6.56%), ketones group (6.47%) and organic acids (3.41%). The PA-PB and CPA-PB that investigated for plant growth promoter and antibacterial activity, respectively, was also further extracted using organic solvent of dichloromethane (DPA-PB) and was analyzed using GS-MS. As shown in Table 5, 48 compounds were identified from DPA-PB with phenols (69.5%), alkyl aryl ethers (9.33%) and ketones (7.76%) were the major constituents while furan and pyran derivatives (3.57%), sugar derivatives (2.85%), organic acids (2.67%) esters (1.81%), aldehydes (1.05%),

TABLE 3. Summary of possible compounds presented in PA of palm kernel shell and pineapple biomass and other lignocellulosic materials

Raw material to produce PA	Absorbance, cm^{-1}	Functional group	Class of compounds
Palm kernel shell	3600-3200	O-H	Phenols, alcohols, water
	1750-1680	C=O	Ketones, aldehyde groups
	1650-1580	O-H	Aromatic group
	1510-1500	C=C	Alkenes
	1470-1350	C-H	Alkanes
	1300-950	C-O, O-H	Different alcohols, phenols, ethers, esters
	900-690	Finger print region	Mono and polycyclic, substituted aromatic
Pineapple biomass	3442	O-H	Polymeric O-H, water impurities
	1634	C=O	Carboxylic acid, ketones, aldehydes
	1416	CH_2 bending	Alkene
	1062	C-O, O-H	Alcohol, phenol, esters, ethers
	695		Aromatic compound
Rice-straw, jute-stick and bagasse (Islam et al. 2012)	3600-3200	O-H	Polymeric O-H, water impurities
	3050-2800	C-H stretching	Alkanes
	1775-1650	C=O	Carboxylic acid, ketones, aldehydes
	1680-1575	C=C	Alkenes
	1550-1475	NO_2	Nitrogenous compounds
	1490-1325	C-H bending	Alkanes
	1300-950	C-O, O-H	Alcohols, phenol, esters, ethers
	900-650		Aromatic compounds
Mangrove (Lee et al. 2011b)	3600-3200	O-H	Polymeric O-H, water impurities
	3500-3200	N-H	Amine compounds
	3000-2800	C-H	Alkanes
	1300-950	C-O, O-H	Alcohols, phenol, esters, ethers
Bamboo (Lee et al. 2011b)	3600-3200	O-H	Polymeric O-H, water impurities
	3000-2800	C-H	Alkanes
	1600-1500	N-O	Nitrogenous compounds
	1600-1400	C=C	Alkenes
	1300-950	C-O, O-H	Alcohols, phenol, esters, ethers

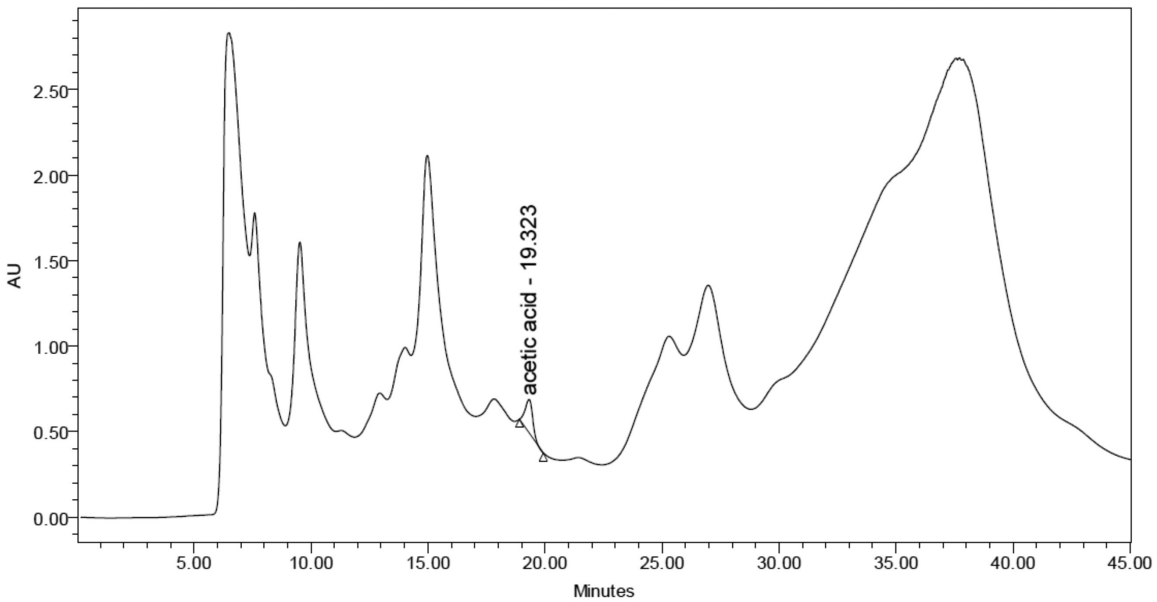

FIGURE 5. HPLC analysis for acetic acid in CPA-PB

TABLE 4. GC-MS result of DPA-PKS

No	Retention time (min)	Name of the compound	Relative content (%)
		Ketones(6.47)	
1	2.40	2-cyclopenten-1-one	0.68
2	2.60	1-(acetyloxy)-2-propanone	0.27
3	2.79	2-piperazinone	0.05
4	3.01	2-methyl-2-cyclopenten-1-one	0.20
5	3.18	2,5-hexanedione	0.14
6	4.12	1-(3-Isobutyryl-bicyclo[1.1.1]pent-1-yl)-2-methylpropan-1-one	0.43
7	4.35	1-[(1H-Pyrrol-2-ylcarbonyl)oxy]-2,5-pyrrolidinedione	0.16
8	4.39	3-methyl-1,2-cyclopentanedione	1.91
9	4.59	2,3-dimethyl-2-cyclopenten-1-one	0.38
10	5.18	3-methyl-cyclohexanone	0.25
11	5.39	1-(2-methyl-1-cyclopenten-1-yl)-ethanone	0.49
12	5.74	3-ethyl-2-hydroxy-2-cyclopenten-1-one	0.57
13	6.67	4,5,6,6a-tetrahydro-2(1H)-pentalenone	0.16
14	9.32	2-allylaminomethylene-5,5-dimethyl-Cyclohexane-1,3-dione	0.12
15	10.08	9-Oxabicyclo[3.3.1]non-6-en-2-one	0.15
16	11.63	1-(3-hydroxy-4-methoxyphenyl)-ethanone	0.22
17	11.75	8-(3-ethoxypropylamino)-1,3-dimethyl-3,9-dihydro- Purine-2,6-dione	0.15
18	13.44	16-Keto-tetrahydrosolasodine	0.21
19	14.35	4-[(2-methoxy ethoxy)methoxy]-5-methyl-tricyclo[6.3.0.0(1,5)]undecan-10-one	0.22
20	15.68	1-(2,4,6-trihydroxy-3-methylphenyl)- 1-Butanone	0.35
21	15.97	Carbazol-2-yl methyl ketone oxime	0.05
		Organic acids (3.41)	
22	2.36	Butanoic acid	0.05
23	3.40	Hexadecanoic acid	0.41
24	4.89	Acetic acid	0.20
25	7.37	Formic acid	0.22
26	10.99	4-hydroxy-3-methoxy-benzoic acid	1.72
27	12.30	4-hydroxy-3-methoxy-benzeneacetic acid	0.82
		Esters/Ethers (1.50)	
28	11.12	Methylparaben	0.76
29	7.48	2,3-Anhydro-d-mannosan	0.51
30	9.27	Hexyl isopropyl ether	0.23
		Alcohols (1.25)	
31	9.38	3-hydroxy-5-methoxy-benzenemethanol,	0.20
32	9.93	5-t-butyl-2-hydroxy-cyclohexanemethanol	0.23
33	12.21	5-tert-butylpyrogallol	0.82
		Alkanes (0.39)	
34	9.04	Dodecamethyl-cyclohexasiloxane	0.21
35	11.81	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	0.06
36	33.45	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-Octasiloxane	0.05
37	34.63	1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- Heptasiloxane	0.03
38	35.52	1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- Heptasiloxane	0.02
39	36.26	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-Octasiloxane	0.02
		Phenols and derivatives (76.42)	
40	3.78	Phenol	47.60
41	3.86	Phenol	0.88
42	3.94	Phenol	0.28
43	3.99	Phenol	0.30
44	4.75	2-methyl-phenol	1.18
45	5.04	4-methyl-phenol	1.80
46	5.33	2-methoxy-phenol	5.60
47	5.65	Maltol	0.45
48	6.02	3-ethyl-phenol	0.33
49	6.18	2,5-dimethyl-phenol	0.42
50	6.46	3-ethyl-phenol	0.19
51	6.50	3,5-dimethyl- phenol	0.24

(continue)

Continued (TABLE 4)

No	Retention time (min)	Name of the compound	Relative content (%)
52	6.72	4-methyl-2-methoxy-phenol	0.24
53	6.84	4-methyl-2-methoxy-phenol	0.37
54	6.94	4-methyl-2-methoxy-phenol	2.10
55	6.99	1,2-Benzenediol	1.45
56	7.64	2,4-Dimethoxytoluene	0.17
57	7.71	2-methoxy-1-methyl-3,5-dinitro- benzene	0.19
58	8.06	3-methoxy-1,2-Benzenediol	1.72
59	8.18	2-methyl-1,4-Benzenediol	0.25
60	8.34	4-ethyl-2-methoxy-phenol	1.59
61	8.50	4-methyl-1,2-Benzenediol	0.22
62	8.62	3-methoxy-phenol	0.14
63	9.49	2,6-dimethoxy-phenol	6.54
64	9.61	4-amino-2-nitro-phenol	0.22
65	9.66	2,6-dimethoxy-phenol	0.37
66	9.76	2-methoxy-4-propyl-phenol	0.33
67	11.06	2-methoxy-6-(2-propenyl)-phenol	0.29
68	11.98	3,4-dimethoxy-benzaldehyde	0.20
69	13.34	2,6-dimethoxy-4-(2-propenyl)-phenol	0.15
70	14.73	2,6-dimethoxy-4-(2-propenyl)-phenol	0.09
71	9.88	2-Methoxybenzhydrazide	0.25
72	10.29	4-Hydroxy-2-methoxybenaldehyde	0.30
		Furan and pyran derivatives (6.56)	
73	2.29	2-methyl-pyridine	0.05
74	2.98	2-ethyl-pyridine	0.06
75	3.07	3,4-dihydro-2H-pyran	0.94
76	3.52	3-Aminopyrazine-1-oxide	2.40
77	4.06	3-methoxy-pyridine	0.88
78	4.54	Pyridine, 4-methyl-, 1-oxide	0.11
79	4.79	2,3-bis(5-methyl-2-furanyl)-2,3-butanediol	0.56
80	4.85	3-Acetyl-1H-pyrroline	0.10
81	4.93	3-Methylpyridazine	0.26
82	5.11	3-Methylpyridazine	0.21
83	6.13	3-Methylpyridazine	0.23
84	8.58	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	0.39
85	3.33	5-methyl-2(5H)-furanone	0.10
86	3.63	4,6-dimethyl-2H-pyran-2-one	0.10
87	4.66	4-methyl-5H-furan-2-one	0.19
		Alkenes (1.80)	
88	2.48	cis-2-1,4-bis(methoxyethoxymethoxy)-cyclopentene,	1.57
89	7.29	1,1-Dimethyl-1-silacyclo-2,4-hexadiene	0.23
		Amide (0.23)	
90	9.07	4-iodo-N-(4-pyridinylmethyl)-1H-Pyrazole-1-acetamide	0.23
		Sulfone (0.42)	
91	7.85	Di-n-decylsulfone	0.20
92	7.79	Methyl p-methylphenylsulfoxide	0.23
		Others (0.87)	
93	2.91	Tetrahydro-thiazole	0.06
94	7.17	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	0.60
95	10.41	1,3-Dimethyl-3-hydroxy-5-methoxyoxindole	0.13
96	12.63	2-[3-Hydroxy-4-methoxyphenyl]-semicarbazide	0.08

TABLE 5. GC-MS result of DPA-PB

No	Retention time (min)	Name of the compound	Relative content (%)
		Ketones (7.76%)	
1	4.58	1-Hydroxy-2-butanone	0.19
2	7.04	2-Cyclopenten-1-one	0.27
3	11.0	2-Methyl 2-cyclopenten-1-one	nd
4	13.8	3-Methyl 2-cyclopenten-1-one	0.36
5	16.3	3-Methyl 1,2-cyclopentanedione	1.44
6	19.6	3-Ethyl 2-hydroxy-2-cyclopenten-1-one	0.36
7	24.4	5-Methyl-2(1-methylethyl)-2-cyclohexen-1-one	0.39
8	30.0	1-(4-Hydroxy-3,5-dimethoxyphenyl)-ethanone	2.55
9	31.2	1,(4-Hydroxy-3-methoxyphenyl)-2-propanone	nd
10	35.1	1,(4-Hydroxy-3-methoxyphenyl)-ethanone	2.20
		Esters (1.81%)	
11	11.3	Butyrolactone	0.99
12	15.0	4-Oxo-methyl pentanoic acid	0.13
13	30.8	Methyl-3-methoxy-4-acetoxybenzoate	0.69
		Organic acids (2.67%)	
14	5.22	Butanoic acid	nd
15	5.24	Propyl-propanedioic acid	nd
16	5.25	Pentanoic acid	0.31
17	33.7	4-Hydroxy-3-methoxybenzene acetic acid	0.84
18	35.6	3,5-dimethoxy-4-hydroxyphenyl acetic acid	1.52
		Aldehydes (1.05%)	
19	5.45	2-Butenal dimethyl acetal	0.67
20	33.9	4-Hydroxy-3,5-dimethoxy benzaldehyde	0.38
		Alcohols (0.9%)	
21	8.47	2-Furanmethanol	0.44
22	17.9	3-Cyclopentene 1,2-diol	0.46
		Furan and Pyran derivatives (3.57%)	
23	11.5	2(5*H*)-Furanone	nd
24	13.4	Dihydro-5-methyl-2(3*H*)-furanone	nd
25	14.1	2*H*-Pyran-2-one	0.15
26	15.4	2,5-Dihydro-3,5-dimethyl 2-furanone	0.20
27	16.9	4-Methyl-5*H*-furan-2-one	0.64
28	19.3	Maltol	1.26
29	20.2	2-Hexyl-tetrahydrofuran	0.19
30	21.8	5-(Hydroxymethyldihydro)-2(3*H*)-furanone	0.64
31	25.7	3-Furancarboxylic acid 4-butyl tetrahydro-2-oxo-methyl ester	0.49
		Sugar derivatives (2.85%)	
32	22.4	1,4:3,6 dianhydro α-D-glucopyranose	2.25
33	23.0	Anhydro D-mannosan	0.60
		Phenol and derivatives (69.5%)	
34	14.7	Phenol	14.8
35	17.5	2-Methyl phenol	0.57
36	18.3	4-Methyl phenol	1.67
37	18.6	2-Methoxy phenol	1.42
38	22.0	2-Methoxy-4-methyl phenol	1.53
39	22.2	1,2-Benzenediol	7.90
40	24.0	3-Methoxy 1,2 benzenediol	4.66
41	24.8	4-Methyl 1,2-benzenediol	1.51
42	26.6	2,6-Dimethoxy phenol	32.9
43	27.4	4-Ethyl 1,3-benzenediol	0.56
44	27.8	2-Ethoxy 5-propenyl phenol	0.76
45	29.2	*p*-Carbomethoxy phenol	1.30
		Nitrogenated derivatives	
46	18.7	1-Methyl 2,5-pyrolidinedione	0.14
		Alkyl aryl ethers (9.33%)	
47	29.0	1,2,4-Trimethoxybenzene	8.91
48	31.2	1,2-Dimethoxy-4-*n*-propyl benzene	0.42

alcohols (0.9%) and nitrogenous compounds (0.14%) were identified as the minor constituents.

Phenols were identified as the main organic components in both DPA-PKS and DPA-PB followed by ketones and furans and pyran derivatives and organic acids. Other components are esters/ethers, alcohols, alkanes, alkenes, amide, sulfone, aldehydes, sugar derivatives and nitrogenated derivatives. This result showed the presence of phenols, ketones and carboxylic groups which attributed to the antifungal activity of PA-PKS as reported by previous studied (Lee et al. 2011b, 2010). Most phenolic compounds have disinfectant properties and Suzuki et al. (1997) suggested that the phenolic compounds of 4-ethyl-2-methoxyphenol and 4-propyl-2-methoxyphenols might have some preservative effects. Previous studies indicated that high contents of organic acids and phenols in the PA indicate that they may correlate with strong inhibition of bacteria (Ma et al. 2011; Wei et al. 2010a). The plant growth might also affected by the presence of organic acids, with acetic acid being one of main components and various forms of alcohol in PA (Mungkunkamchao et al. 2013). The presence of these alcohols, acids and aldehydes in PA could also serve as carbon and energy substrates for soil microorganisms, thus promote the plant growth (Yang et al. 2014). Therefore, further work is in progress for isolation and identification of the antimicrobial and plant growth promoter components in PA from pineapple biomass.

Conclusion

The result showed the potential of pyroligneous acid from palm kernel shell as antifungal and and pyroligneous acid from pineapple biomass as antibacterial and plant growth promoter. Pyroligneous acid from palm kernel shell showed good antifungal properties which can be attributed to the presence of phenols, ketones and carboxylic groups. Acetic acid has been suggested as active compound responsible for the antibacterial properties of pyroligneous acid from pineapple biomass. As for pyroligneous acid of pineapple biomass as plant growth promoter, 2 to 4% (v/v) of pyroligneous acid affected the plant growth in terms of plant height, leaf diameter, root length, fruits weight, leaves number and fruits number. These results can be used as a good preliminary indication for future application of pyroligneous acid for antifungal, antibacterial and plant growth promoter agents.

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