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Influence of Different Extraction Solvents on Lipophilic Extractives of *Acacia* Hybrid in Different Wood Portions

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ABSTRACT

In this study, *Acacia* hybrid was divided into bark, sapwood and heartwood. Each portion undergone Soxhlet extraction according to ASTM Standard D1108-96, D1107-96 and D1110-84 using different polarity solvents; hexane, methanol and hot water. The crude extract of each portion was analyzed to know the chemical components of wood extractives from the extraction solvents. Wood extractives covered in this study were low molecular weight compounds; fatty acids, sterols, glycerides and steryl ester. Gas chromatography analysis was carried out for crude extract of hexane and methanol due to these volatile solvents. Crude extract of hot water was analyzed by High Performance Liquid Chromatography (HPLC) due to its non-volatility. Results shown that total extractives analyzed by Gas chromatography for methanol extract in heartwood was highest, 1.45 mg g^{-1}, followed by bark and sapwood of 1.39 and 0.89 mg g^{-1}, respectively. For hot water extract, heartwood was found to have the most in total extractives, 1.83 mg g^{-1}, followed by bark 1.26 mg g^{-1} and sapwood, 0.82 mg g^{-1}.

Key words: Lipophilic extractives, *Acacia* hybrid, extraction solvents, wood portions

INTRODUCTION

Acacia hybrid is categorizes in the nine selected fast growing species and these species have wide variety of application. *Acacia* species was a fast growing plantation species which used in plantation forestry program among Asia and Pacific countries, due to the rapid growth, less susceptible to disease and high adaptability (Martin, 2004; Kim *et al*., 2008; Khalid *et al*., 2010). This species was a good source of raw material for wood industry. Wood extractives was giving concern due to which it play an important role in making some wood and other lignocellulosic materials incompatible in several application such as cement in making composites (Freire *et al*., 2005). In many hardwood species, lipophilic extractives consists of a complex mixture of compounds such as long-chain aliphatic acids and alcohols, sterols, waxes, sterol esters and glycerides (Freire *et al*., 2005; Sun and Sun, 2001).

Wood extractives also known as lipophilic extractives which are soluble in many organic solvents with low polarity such as hexane and dichloromethane etc. Wood extractives consist of a large variety of low molecular mass compounds which are only a small fraction of wood (Bergelina and Holmboma, 2008). Besides, polar solvent like water can extract hydrophilic salts, sugars, starch and phenolic. Amount and composition of wood extractives is dependent on several

factors such as wood species, age and growth location (Freire *et al*., 2005). In this study, the extraction solvent chose were hexane, methanol and hot water. Hot water was chosen as a extraction solvent due to it can effectively remove the lipophilic extractives as suggested by Sun *et al*. (2003). Although, *Acacia* species is a well known and widely used species, it chemical component of extractives has not been studied. Therefore, the lipophilic extractives of *Acacia* hybrid after extraction using different extraction solvents were the aim of this study.

MATERIALS AND METHODS

Acacia hybrid of 10 years old was taken from Sabah Forestry Development Authority (SAFODA), Kinarut. The dbh portion was taken and divided into bark, sapwood and heartwood. Each portion was flaked into particle size and air dried for two weeks.

ASTM D1108-96 (ASTM, 2007b) was used where, dichloromethane was changed to hexane due to dichloromethane and hexane was non-polar solvents. Besides ASTM D1107-96 (ASTM, 2007a) was used where ethanol-toluene had changed to methanol due to the polarity of methanol and ethanol was almost similar. However ASTM D1110-84 (ASTM, 2007c) was used for hot water extraction. Determination of moisture-free wood was first done before undergone extraction. Two gram wood sample was placed in soxhlet extraction apparatus where filter paper number one was used with 150 mL of extraction solvent and the heating matter was set to number 3 or 4 for solvent to boil for six hour to obtain the first extraction. All the extract was concentrated in rotary evaporator at 60°C to obtain the crude extract. The extracts were kept and stored in refrigerator for further analysis purposes. The same method was repeated for other wood sample.

For extraction using hexane and methanol, the solvent was evaporated to dryness using rotary evaporator. The dried round extraction flask and content was further drying in oven for one hour at 103°C and cool in a dessicator. Continued of drying process until constant weight was obtained. The percentage yield of hexane and methanol soluble matter can be calculated based on ASTM D1108-96 and D1107-96. For extraction using hot water, the extract was filtered using crucible (porosity 1) and was dried in oven at 103°C until the constant weight was achieved and the yield percentage of hot water soluble could be calculated based on ASTM D1110-84.

Gas Chromatography Mass Spectrometer (GCMS) was used to detect *Acacia* hybrid extractives for crude extract of hexane and methanol. The extracts were filtered using PTFE filter membrane before analyzed. This was because filtered extract can reduce noise or disturbance when it was analyzed using GCMS and then affecting the final result. The crude extract was sent to Forest Research Institute of Malaysia (FRIM) and the method of analyzing was according to Ahmad *et al*. (2009). The crude extract was analyzed by Shimadzu GC-14A Gas Chromatography (GC) system equipped with Flame Ionization Detection (FID). BP-X5 capillary column (30 m×0.25 mm id), 0.25 μm film thickness was used. Helium gas was used as a carrier with a flow rate of 25 mL min^{-1} at 600 kPa. The injector and detector temperature were set and maintained at 250°C. Injection was performed in split less mode with the volume of 1 μL. The oven temperature of GC was programmed with the run time of 51.6667 min. The oven temperature of GCMS was programmed as followed;

- 50°C (2.5 min hold) to 150°C at the rate of 15°C min^{-1}
- Increased to 200°C at the rate of 3°C min^{-1}
- Increased to 300°C at the rate of 8°C min^{-1}
- It was kept for another 8 min at 300°C.

Injection temperature was maintained at 250°C, interface temperature at 280°C, quadruple temperature at 150°C and ion source temperature at 230°C. Injection was performed in split less mode and the volume used was 1 μL. The mass spectra of compounds in samples were obtained by Electron Ionization (EI) at 70 eV, the detector operated in scan mode from 20 to 600 amu. Compounds were identified by direct comparison of their chromatograms retention time with those of authentic compounds. Free fatty acids were quantitatively determined relative to the palmitic acid standard, resin acids relative to abietic acid, sterols relatives to sitosterols, steryl esters relative to cholesteryl palmitate, glycerides relative to 1,2-dipalmitoyl-sn-glycerol for diglycerides and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol for triglycerides as standard compound. Besides, the identification of *Acacia* hybrid extractives was performed by the NIST software library.

Due to unsuitability of hot water extract analyzed by Gas Chromatography, HPLC was chosen for analysis of wood extractives contained in the hot water extract. HPLC analysis of hot water extract was carried at Forest Research Institute of Malaysia (FRIM). The standard compounds were still remaining the same as gas chromatographic analysis compounds. Each sample of hot water extract which namely bark, sapwood and heartwood were dried completely and then 1 mL water was added to each sample. The resulting solution was filtered through a membrane filter (pore size 0.45 μm). An aliquot of 800 μL each was diluted with 400 μL water prior to analysis.

The sample was analysed by means of a HPLC system (Waters Delta 600 with 600 Controller) with photodiode array detector (Waters 996). A Phenomenex-Luna (5 μm) column was used (4.6 mm i.d×250 mm) and for elution of the constituents, two solvents denoted as A and B were employed. A was 0.1% aqueous formic acid, B was 0.1% formic acid in acetonitrile. Initial conditions were 85% A and 15% B with a linear gradient reaching 25% B at t = 12 min. This was maintained for 10 min after which the programme returned to the initial solvent composition at t = 25 min and continued for 10 min. The flow rate used was 1.0 mL min^{-1} and the injection volume was 10 μL. The retention times and UV spectra of major peaks were analysed.

RESULT AND DISCUSSION

The yield and chemical composition of lipophilic extractives were shown in Table 1. The yield of extraction was calculated based on the dry basis of the extract. The chromatograms of hexane and methanol were shown in Fig. 1-6. Generally, range of retention time 5-15 min was fatty acids, sterols was analyzed at after 15 until 24 min of the retention time, while steryl ester and glycerides were analyzed after 28 min for the retention time. There were several group of free fatty acid that had been found in the extract. Some trace amounts of other fatty acids which had been denoted as C12:0, C14:0, C15:0 and C17:0. C12:0 referred to decanoic acid, C14:0 referred to tetradecanoic acid, C15:0 referred to Pentadecanoic acid and C17:0 referred to Heptadecanoic acid.

Hexane and methanol extract were analysed by GC after silylation on the extract. Compounds of *Acacia* hybrid were separate by GCMS with a medium polarity column BP-X5. The compounds were compared through the retention time which was similar to the standard compounds. GCMS system is very useful for analysing fatty acids and sterols in the extract (Wallis and Wearne, 1999). Hexane had been chosen to extract lipophilic extractives from the samples due to its non polar organic solvent (Qin *et al*., 2009). It showed that Hexane extract fatty acid the most in heartwood of *Acacia* hybrid that was 0.67 mg g^{-1}, followed by bark that was 0.60 mg g^{-1} and sapwood had low content of fatty acids which was 0.30 mg g^{-1}. Methanol also extract similar amount of fatty acid where the heartwood and sapwood gave high amount of fatty acid that were 0.95 and 0.66 mg g^{-1}, respectively and bark contained low amount of fatty acids in methanol extract that was 0.57 mg g^{-1}.

Table 1: Yield (%) of extraction and chemical composition (mg g^{-1}) soluble in hexane and methanol of *Acacia* hybrid

	Bark	Sapwood	Heartwood	Bark	Sapwood	Heartwood
Yield	5.71	2.12	3.24	6.48	4.09	5.31
Free Fatty Acid/ Resin						
Decanoic acid, C10:0	0.01	0.01	0.03	0.01	a	0.11
Hexadecanoic acid, C16:0	0.01	0.01	0.03	0.02	a	0.02
Octadecanoic acid, C18:0	0.02	0.02	0.01	0.01	0.07	0.07
Linoleic acid, C18:2	0.01	0.01	0.01	0.12	0.10	a
Docosanoic acid, C22:0	0.05	0.02	0.05	0.02	0.08	0.04
Tetracosanoic acid, C24:0	0.15	0.02	0.12	0.11	0.22	0.13
Others 1	0.35	0.21	0.42	0.28	0.19	0.58
Sum fatty acids	0.60	0.30	0.67	0.57	0.66	0.95
Glycerides						
Mono-glycerides	a	0.02	a	a	0.01	0.01
Diglycerides	0.04	0.13	0.06	0.01	0.05	0.05
Triglycerides	0.38	0.40	0.51	0.23	0.35	0.42
Sum glycerides	0.42	0.55	0.57	0.24	0.41	0.48
Sterols						
Ergosterols	a	0.03	0.01	0.04	a	0.03
Sitosterols	0.16	0.02	a	0.02	0	0.01
Stigmasterols	0.33	0.24	0.23	0.27	0.16	0.24
Others	0.10	0	0.01	0.04	a	0.02
Sum sterols	0.59	0.29	0.25	0.37	0.16	0.30
Steryl ester	0.19	0.23	0.17	0.15	0.12	0.15
Total	1.80	1.37	1.66	1.33	1.35	1.88

Note: a = Trace amount, 1 = C12.0, C14.0, C15.0, C17.0

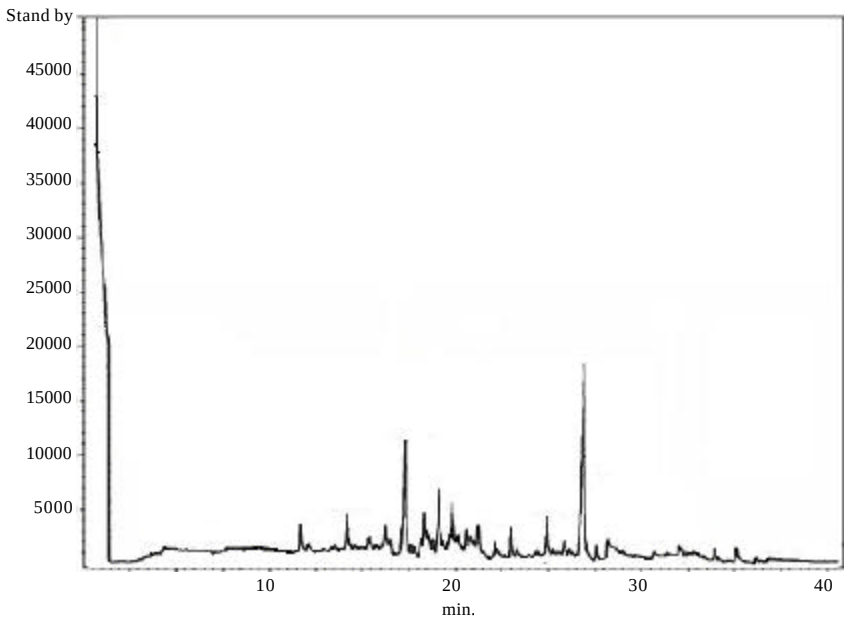

Fig. 1: Chromatogram of *Acacia* hybrid extractives for bark extracted by hexane

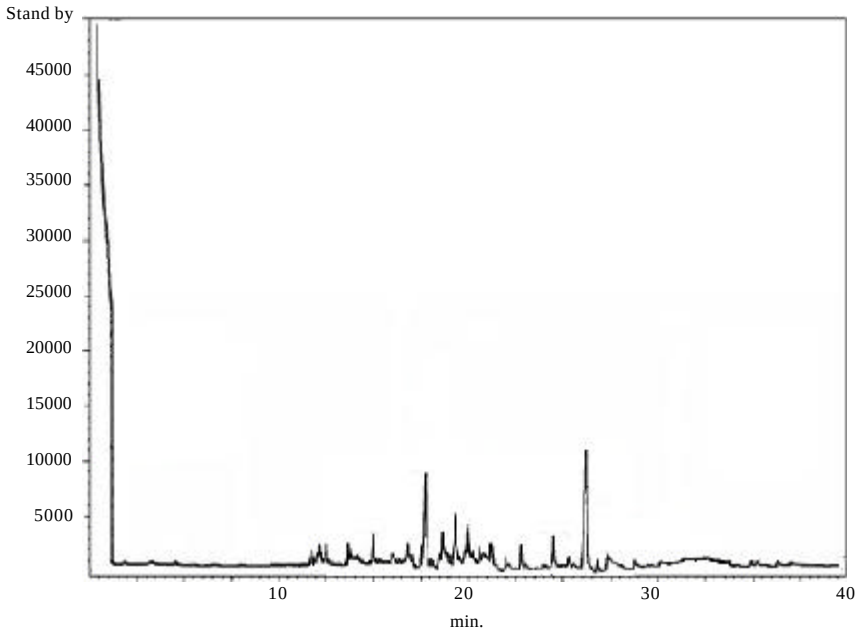

Fig. 2: Chromatogram of *Acacia* hybrid extractives for Sapwood extracted by hexane

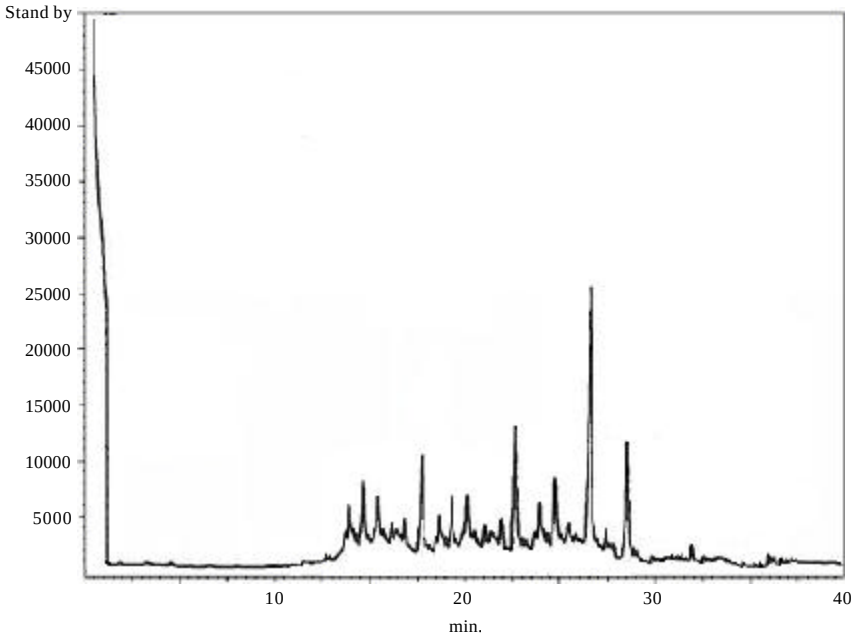

Fig. 3: Chromatogram of *Acacia* hybrid extractives for Heartwood extracted by hexane

Sterols were also an important class of lipids presented in *Acacia* hybrid. There were three type of sterols identified namely ergosterol, sitosterols and stigmasterols. Generally, bark had high content of sterols that was 0.59 mg g^{-1}, followed by heartwood and sapwood which were 0.29 and 0.25 mg g^{-1}. Bark undergone methanol extraction contained high amount of sterols that was 0.37 mg g^{-1}, followed by heartwood that was 0.30 mg g^{-1} and sapwood contained low amount of

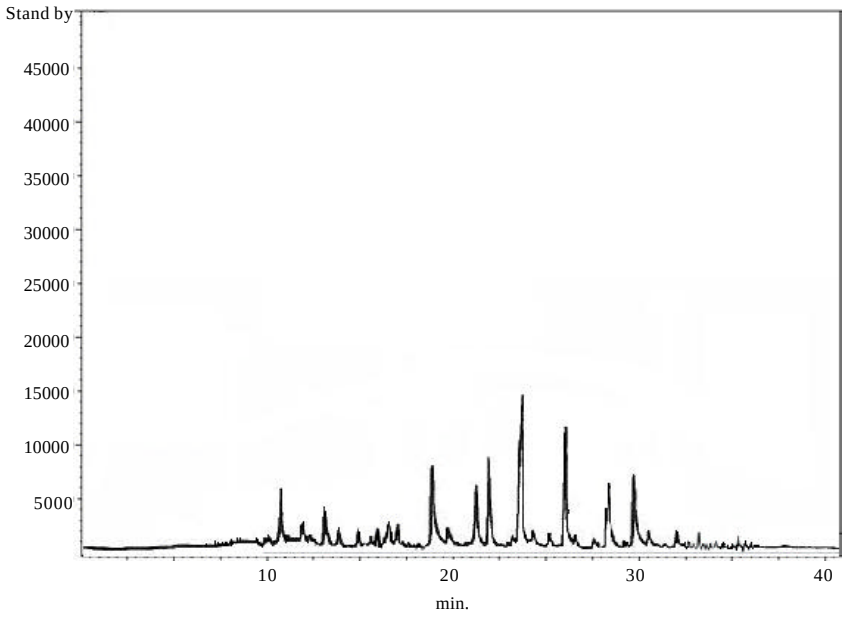

Fig. 4: Chromatogram of *Acacia* hybrid extractives for bark extracted by methanol

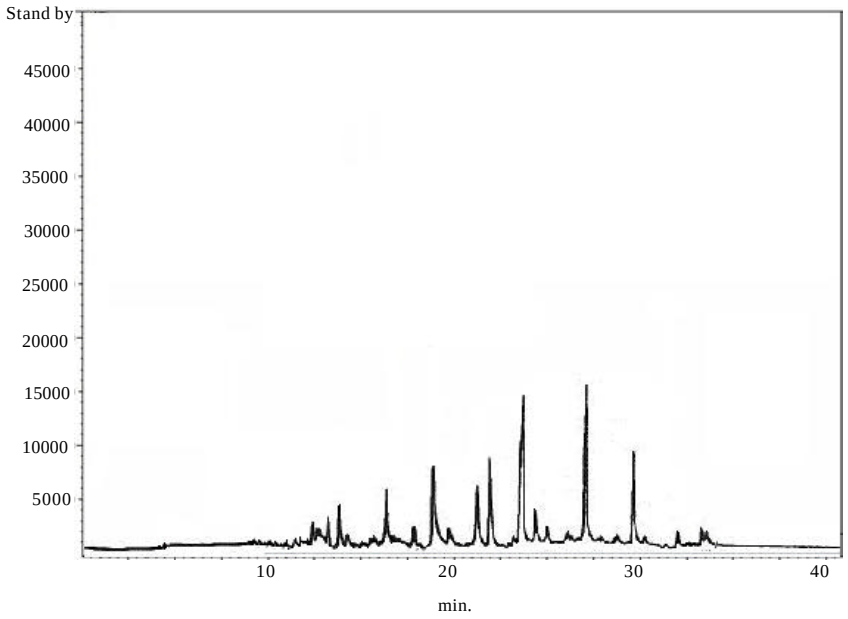

Fig. 5: Chromatogram of *Acacia* hybrid extractives for sapwood extracted by methanol

sterols that was 0.16 mg g^{-1}. In this study, hot water extraction show the lowest value of steryl ester extracted among the three solvents, while hexane and methanol show similar amount. It agreed by Pietarinen *et al.* (2004) where, they were studying on *Acacia mangium* and *Acacia crassicrapa.*

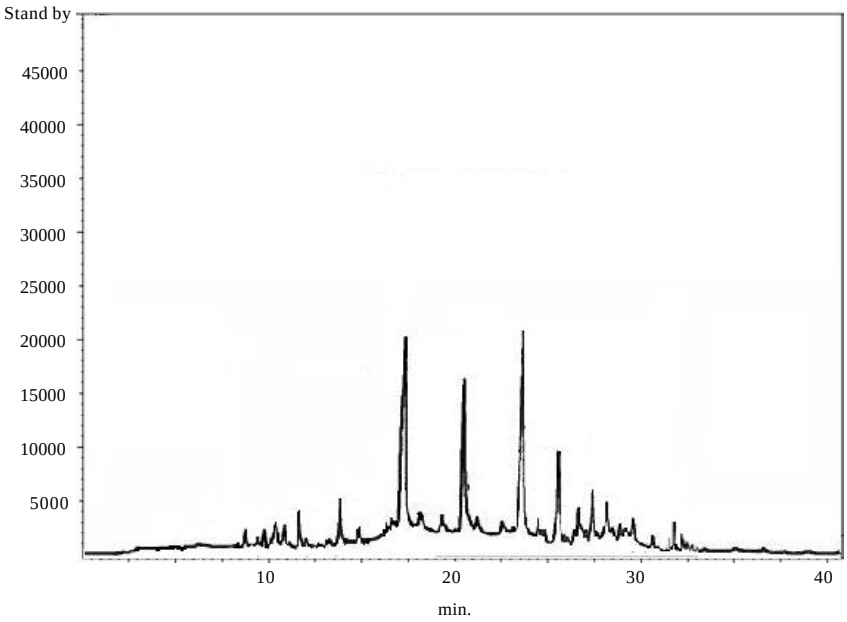

Fig. 6: Chromatogram of *Acacia* hybrid extractives for heartwood extracted by methanol

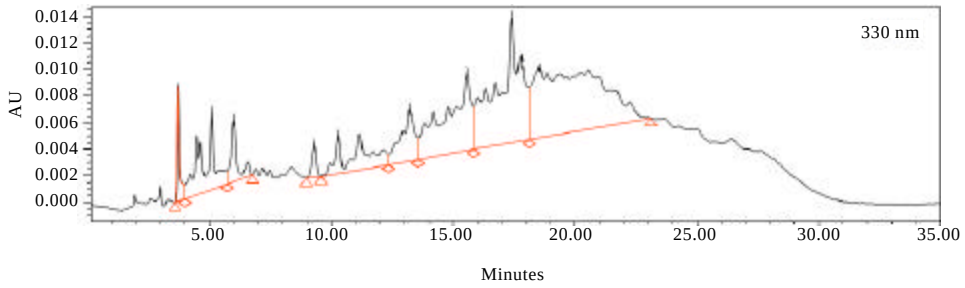

Fig. 7: HPLC chromatogram of *Acacia* hybrid extractives for bark of hot water extraction

Glycerides consist of mono, di and triglycerides in *Acacia* hybrid extractives. Triglycerides is generally produced as storage reserve of energy and carbon skeletons for growth and development since they are twice as efficient for metabolite energy as either carbohydrates or protein (Sun and Sun, 2001). Hexane extract showed similar amount of glycerides extracted in all wood portions which were bark, sapwood and heartwood. Methanol extract had slightly different of glycerides compounds where sapwood and heartwood contribute the highest amounts which were 0.41 and 0.48 mg g^{-1}, respectively, bark undergone methanol extraction only gave a small amount of glycerides which was 0.24 mg g^{-1}.

HPLC chromatograms for hot water extracted bark, sapwood and heartwood were presented in Fig. 7-9. The profiles obtained had a good resolution for identify the extractives compounds. Each standard compounds were injected before analysed the extract. Then, peak obtained were direct compared to the standard compounds obtained. The sterols and steryl ester obtained were in trace amount which had found at the beginning. Peak obtained at 5 to 10 min. Retention time (RT) was

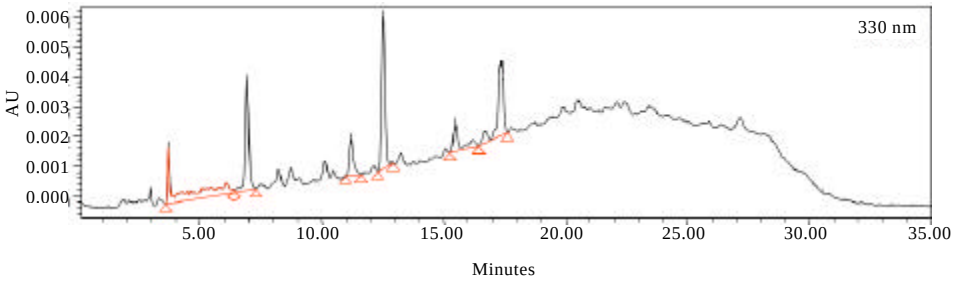

Fig. 8: HPLC chromatogram of *Acacia* hybrid extractives for heartwood of hot water extraction

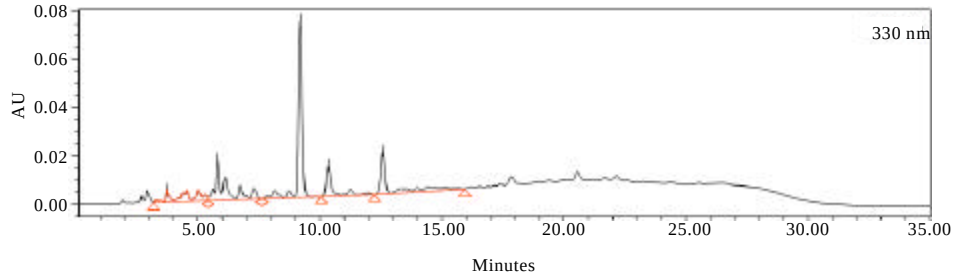

Fig. 9: HPLC chromatogram of *Acacia* hybrid extractives for sapwood of hot water extraction

Table 2: Yield (%) of extraction and chemical composition (mg g^{-1}) soluble in hot water of *Acacia* hybrid

	Bark	Sapwood	Heartwood
Yield	8.23	5.15	6.45
Free Fatty Acid/Resin			
Decanoic acid, C10:0	a	a	0.05
Hexadecanoic acid, C16:0	a	a	0.01
Octadecanoic acid, C18:0	0.01	0.01	0.04
Linoleic acid, C18:2	0.04	a	0.02
Docosanoic acid, C22:0	0.03	0.02	0.03
Tetracosanoic acid, C24:0	0.03	0.1	0.09
Others 1	a	0.07	0.13
Sum fatty acids	0.11	0.2	0.37
Glycerides			
Mono-glycerides	a	a	0.01
Diglycerides	0.01	0.04	0.03
Triglycerides	0.1	0.02	0.11
Sum glycerides	0.11	0.06	0.15
Sterols			
Ergosterols	0.02	0.01	0.02
Sitosterols	a	0	a
Stigmasterols	0.18	0.13	0.13
Others	0.03	a	0.02
Sum sterols	0.23	0.14	0.17
Steryl ester	0.04	0.02	0.03
Total	0.49	0.42	0.72

Note: a = Trace amount, 1 = C12.0, C14.0, C15.0, C17.0

the fatty acids for bark, sapwood and heartwood. The amount analysed by HPLC had shown in the Table 2. Sterols and steryl ester analysed from heartwood were 0.17 and 0.03 mg g^{-1}, the content of sterols and steryl ester for bark were 0.23 and 0.04 mg g^{-1}, respectively, while sapwood contained little amount of sterols and steryl ester which were 0.14 and 0.02 mg g^{-1}, respectively.

The amount of fatty acids were low when extraction using hot water among these three extraction solvents. The content of fatty acids was highest in heartwood which undergone hot water extraction that was 0.37 mg g^{-1}, followed by sapwood that was 0.20 mg g^{-1} and bark contained the lowest of fatty acids contents that was 0.11 mg g^{-1}. Glycerides analysed from hot water extract were very low. However, heartwood extracted by hot water was highest among the portions which were 0.15 mg g^{-1}, followed by bark which was 0.11 mg g^{-1} and sapwood was only content 0.06 mg g^{-1} of glycerides.

CONCLUSION

Lipophilic extractives extracted from *Acacia* hybrid composed of free fatty acid, glycerides, sterols and steryl ester. Different polarity of extraction solvents can extract different amount of extractives from the wood. It should be note that these kind of extractives should be proper extract in order to produce a high quality pulp. Besides, the existence of lipophilic extractives can affect the papermaking process which mainly cause by pitch deposition. There was a large variety of wood extractives found inside *Acacia* hybrid. Bark, sapwood and heartwood of *Acacia* hybrid in breast height portion were studied on wood extractives. It was found that low molecular weight of extractives such as sterols, fatty acids, glycerides and steryl ester were found highest from wood portions, where bark was 11.57%, sapwood was 4.58% and heartwood was 11.54%. Besides, different polarity solvents were able to extract different percentage of extractives, where hexane, methanol and hot water were gave different amount of extractives. Different amount of extractives in each wood portion were observed. Bark extracted by hexane was 5.71%, methanol extracted bark was 6.48% and hot water extracted bark was 8.23%. The extractives of sapwood extracted by hexane was 2.12%, methanol extracted sapwood was 4.09% and hot water extracted sapwood was 5.15%. However, extractives of heartwood extracted by hexane was 3.24%, methanol extracted heartwood was 4.09% and hot water extracted heartwood was 6.45%.

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