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RESEARCH ARTICLE

QUALITATIVE ANALYSIS OF *EUSIDEROXYLON ZWAGERI* TEIJSM AND BINN SEED BY GC-MS AND LC-MS

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Cis-Feruloyltyramine, Iron Wood

Abstract

Background: *Eusideroxylonzwageri* is an endemic tree in certain areas of Borneo and Sumatra. Limited reports are available for the ethno medical use, phytochemical constituents, pharmacological activities of its seed. Objective: The present study was focused on the phytochemical constituents of *E.zwageri* seed extracts, polar (aqueous) and nonpolar (Ethanol and Ethyl Acetate) compounds.

Methods: GC-MS and LC-MS techniques were used to establish the phytochemical profiles of the studied extracts.

Result: β -Asarone was detected as single dominant component of the ethanol and ethyl acetate seed extract from *E.zwageri*. The GC-MS result of the ethanol and ethyl acetate extracts exerted considerable β -Asarone, with 82.44 and 73.05 area % respectively. Additionally, four main flavonoids were found in the aqueous seed extract, namely N-cis-Feruloyltyramine, 3'-O-Methylviolanone and two chromones N-cis-Feruloyltyramine was detected mainly in ethanol seed extract, with 148.787 response. 3'-O-Methylviolanone was detected in water and ethyl acetate seed extracts, with 58.178 and 55.730 response respectively. Two chromones were recognized. First, 3-(4'-Hydroxy-benzyl)-5,7-dihydroxy-6-methyl-8-methoxy-chroman-4-one was detected in ethyl acetate extract of the seed with 94.652 response. Second, 6-Hydroxy-2-[2-(4'-methoxyphenyl) ethyl] chromone was detected in ethanol extract of the seed with 31.132 response.

Conclusion: Findings from the study tend to support the idea that the seed of *E.zwageri* can be utilized as effective bio-resources for designing novel health-promoting products or ingredients. Seed of *E.zwageri* is a resource for α and β Asarone, O-Methylviolanone, N-cis-Feruloyltyramine, 3-(4'-Hydroxy-benzyl)-5,7-dihydroxy-6-methyl-8-methoxy-chroman-4-one, and 6-Hydroxy-2-[2-(4'-methoxyphenyl)ethyl] chromone.

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Introduction:-

Iron wood tree is found and characteristic of ironwood forest. It is distributed in certain areas in Sumatra, Borneo, and Southern Philippines. In Borneo, *E.zwageri* (Lauraceae) is common in lowland dipterocarp forest. Ironwood

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trees can form a distinctive monodominant forest. The canopy of about 30-35 meter height. The seed is like a rugby ball. It is the largest of all recorded dicotyledon seeds (Figure 1) [1,2]. The wood of this tree is well known for its high quality and good for wooded house construction. The leave of *E. zwageri* leave is often used traditionally as traditional medicine, such as antipyretic, gynaecological problems, and counter-poison [3].The seed is used traditionally for enhancing hair growth and black coloring shampoo.

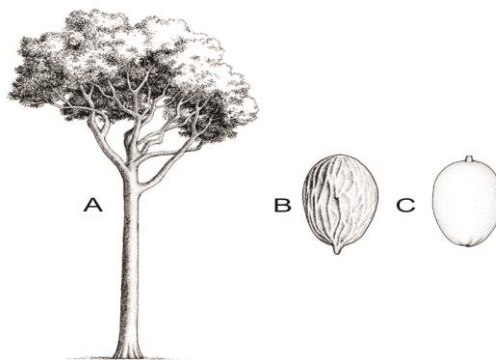


Figure 1:- *Eusideroxylonzwageri* (with permission [2]). A) Tree, B) Fruit, C) Seed.

The objectives of this study were to evaluate the phytochemical profile of the seed extracts of iron wood with use of GC-MS and LC-MS. The studied seeds were extracted with water (cognation), ethanol and ethyl acetate.

Material and Methods:-

Materials

Commercial seeds were bought from the local market in Palangka Raya, Kalimantan, Indonesia in October 2019.

Extract preparation

Seed was cut into small pieces and then cognated in aquadest at 80°C for five hours. For ethanol and ethyl acetate extract, the seed powders were macerated for overnight and then filtered and evaporated.

UV-VIS Scanning

The extracts were examined by UV-VIS spectrophotometer for proximate analysis. The extracts were scanned in the wavelength ranging from 200 – 700 nm using a Spectrophotometer Libra S22, which was used to detect the characteristic peaks. Each and every analysis was repeated in triplicate for the spectrum confirmation.

LC-MS analysis

The extracts (2 -5 uL) were injected to ultra-performance liquid chromatography (UPLC) from Waters Accquity I-Class FTN with column oven along with a mass spectrophotometer Time of Flight of the Waters Xevo G2-S QTOF with Positive and negative Electrospray ionization (ESI). The mobile phase was formic acid 0.1% in H₂O and formic acid 0.1% in acetonitrile. The flow rate was 0.60 mL/min., column temperature was 40 °C. The energies used to obtain the ions were cone at 40 V, capillary at 2.0 KV and collision energy varying between 15 and 40 V using nitrogen as nebulizer and desolvation gas, argon as collision gas. Identification of the Mass spectra were done by comparing with the UNIFI library.

GC-MS analysis

Ethanol extracts were examined using GC-MS on a GC Agilent 7890B equipped with a MS Agilent 7000 and with an Agilent HP-1MS (30mx 0.25 mm inner diameter, 0.25 µm film thickness). The injector was operated at a 310 °C in split mode (1:33) at a head pressure of 1.987 psi of Helium. The temperature program started with an isothermal step at 100 °C for 5 min. The temperature was then raised to 300 °C at a rate of 10 °C/min and held for 3 min. The GC was coupled to a quadrupole mass spectrometry MS Agilent 7000. The chromatograms were identified by comparing their mass spectra and retention index (RI) with the values of our group internal library, NIST and literature.

Results:-

UV-VIS scanning of the aqueous extract showed that there one main peak at 270-279 nm. The ethanolic extract showed two main peaks at 270 nm and 280 nm. Ethyl acetate extract showed at least two main peaks at 254 and 260 nm.

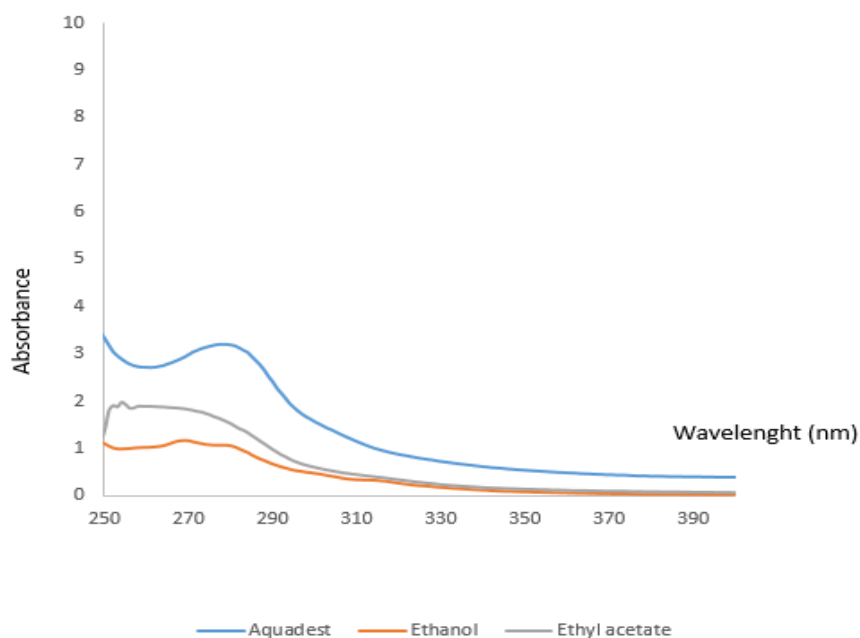


Figure 2:- UV VIS Scanning of the aqueous, ethanol and ethyl acetate seed extract of *E.zwageri*

GC-MS analysis of the main component of the ethanol and ethyl acetate extracts was β -Asaron (Table 1, Figure 3), with 82.44 and 73.05 % area respectively.

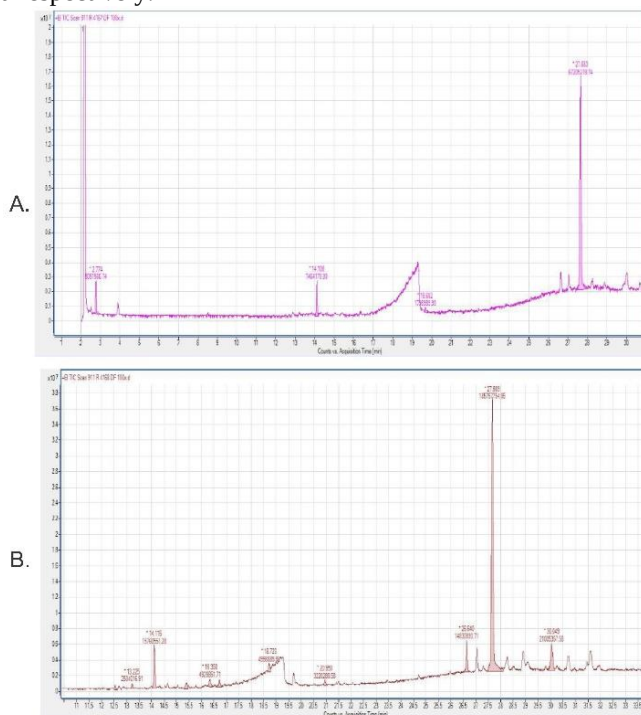


Figure 3:- Chromatograms of GC-MS. A) Ethanol extract, B) Ethyl Acetate extract.

Table 1:- List of compounds that detected by GC-MS analysis.

Compounds	RT (min)	Area (%)	
		Ethanol	Ethyl Acetate
Glycerin	3.903	6.20	
Alfa Copaene	12.533		0.80
Caryophyllene	13.225		0.98
Methyl-5-methylene-8-(1-methylethyl)-[S-(E,E)]-1,6-cyclodecadiene	14.123	9.16	6.20
2-Methylene-6,8,8-trimethyltrycyclo[5.2.2.0(1,6)undecan-3-ol	15.405		1.76
Columbin	16.365		1.94
6-isopropenyl-4.8a-dimethyl-1,2,3,5,6,7,8,8a octahydro-napthalene-2-ol	16.726		1.55
(Z, Z, Z)-9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[trimethylsilyl]oxy]methyl]ethyl ester	18.738		3.94
1-Monolinoleoylglycerol trimethylsilyl ether	19.690	2.21	
Columbin	20.965		1.27
2-Indazol-2-ylphenylamine	26.632		5.83
β-Asarone	27.669	82.44	73.05
7-Acetoxy-7-desacetamino-10-methio-10-desmethoxy-colchicine	30.049		8.26

Four main constituents detected by LC-MS positive mode. First, N-cis-Feruloyltyramine was found mainly in ethanol seed extract. Second, 3'-O-Methylviolanone is detected in aqueous and ethyl acetate seed extracts. Third, 6-Hydroxy-2-[2-(4'-methoxyphenyl)ethyl] chromone was detected in ethyl acetate seed extract. Fourth, 3-(4'-Hydroxy-benzyl)-5,7-dihydroxy-6-methyl-8-methoxy-chroman-4-one was detected in ethanol seed extract (Table 2, Figure 4). The negative mode of LC-MS detected Cnidimol F and Buddlenoid A with low response in aqueous extract, and very low or no response in the ethanol and ethyl acetate seed extract (Table 2, Figure 5).

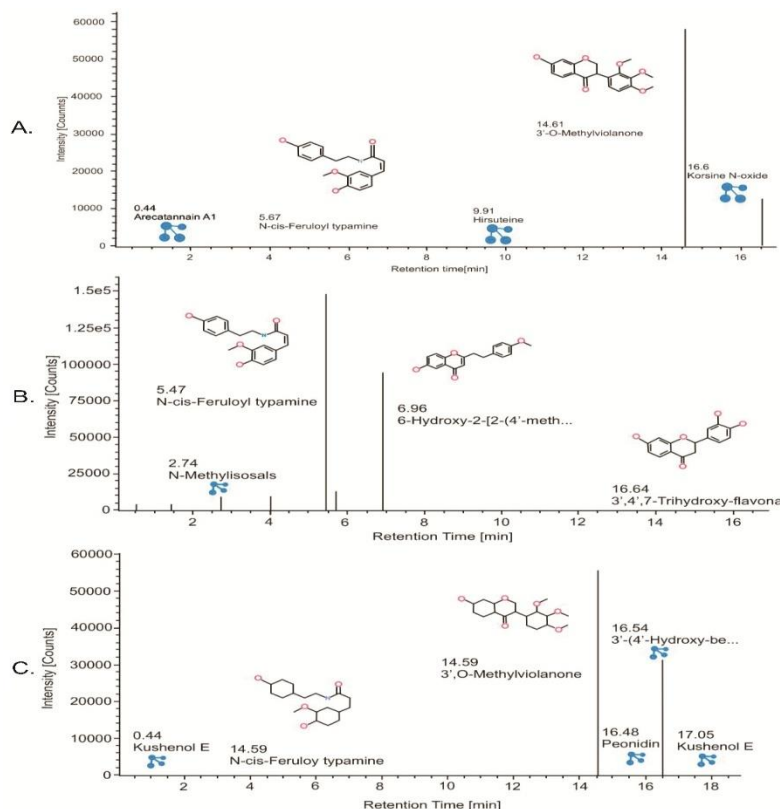


Figure 4:- LC-MS Chromatograms of Aqueous (A), Ethanol (B), and Ethyl Acetate (C) seed extract of *E. zwageri* (positive mode).

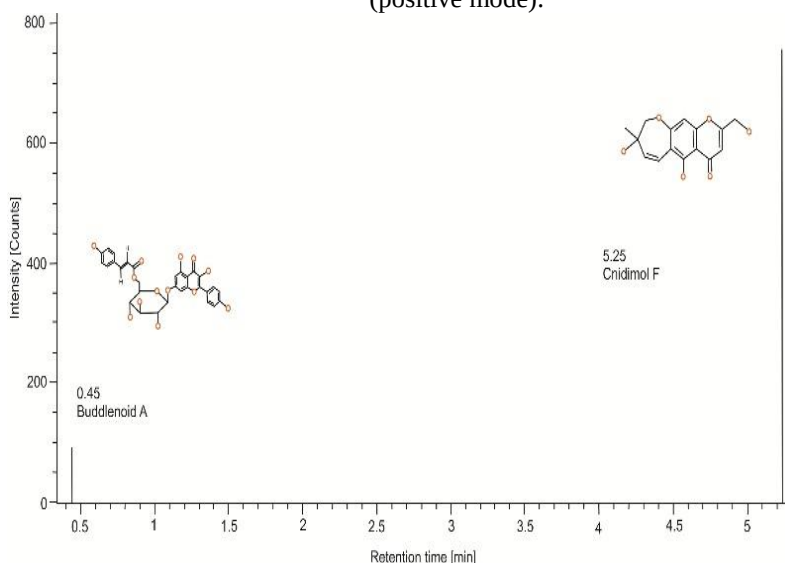


Figure 5:- LC-MS negative mode for the aqueous extract.

Table 2:- List of compounds that detected by LC-MS.

Compounds	Mode	Aqueous	Ethanol	Ethyl Acetate
N-cis-Feruloyltyramine	+	2.185	148.787	1.383
3'-O-Methylviolanone	+	58.178		55.730
3-(4'-Hydroxy-benzyl)-5,7-dihydroxy-6-methyl-8-methoxy-chroman-4-one	+			31.132
6-Hydroxy-2-[2-(4'-methoxyphenyl)ethyl] chromone	+		94.652	
N-Methylisococlaurine	+		13.628	
Korsine N-oxide	+	12.656		
N-Methylisosalsoline	+		9.604	
Isosalsoline	+		9.337	
Adenosine	+		4.288	
Cuscohygrine	+		3.991	
5,8-Dihydroxy-2-(2-phenylethyl) chromone	+		1.903	
3'-Hydroxy-2,4,5-trimethoxydalbergiquinol	-			1.783
d-Isoboldine	+	1.013		
Peonidin	+			961
4,7-Dihydroxy-2,3,6-trimethoxyphenanthrene	-			873
Cnidimol F	-	814		
4',5,6,7-Tetramethoxy-flavone	+			798
3',4',7-Trihydroxy-flavanone	+		660	
Curcumin	+	252		

In order to know whether there is β -Asaron in aqueous extract, a partition of aqueous extract was carried out with ethyl acetate. And then the water-ethyl acetate extract was analysed with GC MS. The result showed that there was no β -Asaron detected in the aqueous-ethyl acetate extract. But, two detected constituents in the water-ethyl acetate extract were Cyclopentane, (1-methylethyl)- (syn. Cyclopentane, isopropyl-, 18.81%) and elemicin (30.58%). LC MS positive mode analysis of the aqueous and ethyl extract showed the presence of 3'-O-Methylviolanone, Catechin, and 3'-O-Angeloylhamadol. The LC/MS negative mode analysis showed the presence of Catechin, Procyanidin B1, and 3-Hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-2-propanone (Table 2, Figure 6).

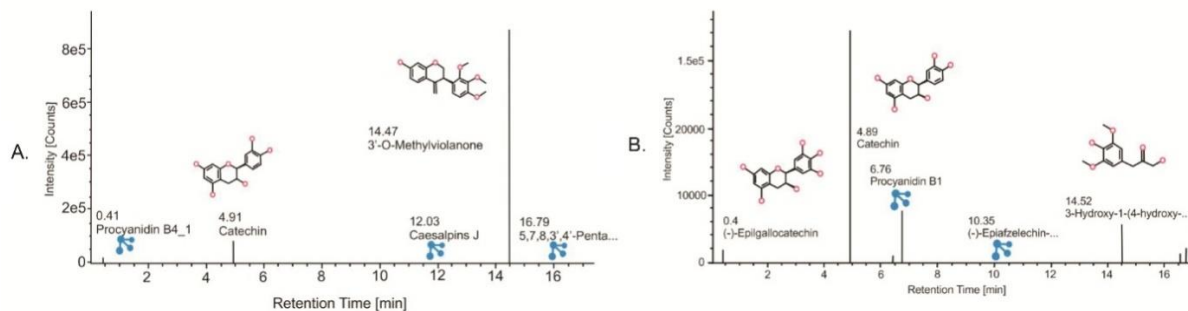


Figure 6:- LC-MS of the aqueous-ethyl acetate partition. A) Positive mode, B) Negative mode.

Discussion:-

Many research on the phytoconstituents of *E.zwageri* are done with extract from the wood, stem bark, and leave. These extracts contain many phytochemical constituents that belongs to arylpropanoids, alkaloids, flavanoids and terpene, especially condensed tannins and lignins[4,5]. So far no report on the phytochemical profile of the seed of *E.zwageri*. Therefore, this is the first report on the chemical constituents of the seed. The chemical profile of the seed is totally different with the other plant parts. Many compound detected from wood, stem bark, and leave extracts are not found in the seed extract.

Seed of *E. zwageri* contain β -Asarone as its dominant compound in ethanol extract. Therefore, seed of *E. zwageri* is a good resource of β -Asarone as an important medicinal ingredient. β -Asarone is known for its significant pharmacological effects, such as on the central nervous system, attenuation of neuronal autophagy or reducing autophagy, treating diabetes, managing cognitive impairment such as Alzheimer's disease[6]. β -Asarone is able to improve the impairment of spatial memory and reduced β -Amyloid induced neural apoptosis in hippocampus and to inhibit the proliferation and apoptosis of lymphoma cell[7,8].

Another four main compounds of seed from *E. zwageri* are N-cis-Feruloyltyramine, 3'-O-Methylviolanon, and two chromones. These compounds are polar compounds and detected in aqueous extract of the seed from *E. zwageri* (Figure 7). N-cis-Feruloyltyramine is a phenolic amide that can be detected only in ethanol seed extract of *E. zwageri*. It exhibits modest inhibitory activity on LPS-activated nitric oxide production and alpha-glucosidase[9,10]. 3'-O-methylviolanon is a kind of flavonoid that can be detected either in aqueous or ethyl acetate extracts. This compound has significant anti-inflammatory activity. Not many information available about the medical use of this compound. 3'-3'-O-Methylviolanon is reported as strong inhibitor of the superoxide formation. Since the reactive oxygen species and the lysosomal enzyme are generated by activate neutrophils, the uncontrolled process may cause the neutrophils to affect the adjacent cells. The pathogenesis of many diseases probably involve this uncontrolled process[11].

Two chromones are not detected in aqueous seed extract. 3-(4'-Hydroxy-benzyl)-5,7-dihydroxy-6-methyl-8-methoxy-chroman-4-one is found in ethanol extract. Meanwhile, 6-Hydroxy-2-[2-(4'-methoxyphenyl) ethyl] chromone is found in ethyl acetate extract and not found in aqueous extract. These compounds are the colouring constituents in the seed of iron wood. 3-(4'-Hydroxy-benzyl)-5,7-dihydroxy-6-methyl-8-methoxy-chroman-4-one has ability to diminish the oxidative stress. This compound is also reported for its cytotoxic activity against the human cancer cell lines. Therefore, It may lead to reduce the developing cancer[12,16]. 6-Hydroxy-2-[2-(4'-methoxyphenyl) ethyl] chromone possess potential anti-inflammatory properties[13]. Several chromones are reported for its potential pharmacological bioactivities, such as antiviral agent, anti inflammation and anticancer[14,15,16]. Pharmacological activities of chromones containing extracts from ironwood seed should be investigated.

Conclusion:-

This is the first study reporting β -Asarone as main constituent in ethanol and ethyl acetate extracts of the seed from *Eusideroxylonzwageri*. Another dominant compounds are N-cis-Feruloyltyramine, and 3'-O-Methylviolanon and two chromones. This preliminary observation suggests a potential medicinal use of the seed of *E.zwageri*. It is anticipated that the finding of the above chemical constituents in this seed will open new avenues for research and contribute towards establishing primary data on these species for designing novel phytopharmaceuticals.

Acknowledgement:-

None

Conflict of interest:-

The authors declare that there is no conflict of interest in writing this review.

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