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A COMPARATIVE EVALUATION OF THE ANTIOXIDANT ACTIVITY OF LOCAL PLANTS ORIGINATED FROM SUMENEP REGENCY, EAST JAVA, INDONESIA

I. Ismawati [1], R. Yuniastri [1], N. Huzaimah [2], T. Estiasih [3], E. Martati [3], D. Tarmadi [4,7], W. Fatriasari [5,7], E. T. Arung [6,7] and M. Ismayati [5,7]✉

ABSTRACT

This is the first study on the antioxidant activity and chemical analysis of leave extract originating from the Sumenep regency, Indonesia. The aim of the present study was to analyze the antioxidant activity from leaf extracts of *B. pilosa*, *H. corymbosa*, and *P. pellucida* and evaluate their chemical components by PyGCMS. The higher yield extracts were B-1 and P-1, about 19.33% and 20.27%. The result of the DPPH test revealed that B-1 was the highest antioxidant properties, with an IC_{50} value of about 6.43%. The antioxidant properties were affected by the high phenolic content of B-1 (112.20%) and the absence of hydrolyzed tannins. The H-1 has higher phenolic content than B-1 but is contrarily in antioxidant activity. The carbohydrates derivatives were easier extracted in aquadest extract has decreased antioxidant activity at H-1. In addition, as reported by GCMS, PyGCMS also showed terpenoid compounds and not only polyphenols (tannins and flavonoids). The interaction between compounds in plant extracts plays a more critical role in antioxidant activity than certain compounds. B-1 extract has the potential as an antioxidant additive in the food, cosmetic, or advanced materials industry.

Keywords: antioxidant, *Bidens pilosa*, DPPH, *Hedyotis corymbosa*, *Peperomia pellucida,* phenolic,

INTRODUCTION

Indonesia has diverse plants, ethnicity, and community culture, with approximately 143 million hectares of tropical rainforests, a supportive tropical climate, and almost 80% of the world's medicinal plants.[1] Furthermore, thousands of flora have been identified and used by ethnic communities because of their properties to cure many diseases.[2] The knowledge of using plants as traditional medicine is passed to different generations in the Indonesian communities. A previous study has shown that the knowledge of the Madurese community about the use of plants as herbs comes from their local awareness. The plant of *B. pilosa, P. pellucida* L., and *H. corymbosa* L. were used by the Madurese community as traditional medicines (anti-fever, antidiabetic, and antitumor)[3]

The characteristics and uses of this plant contribute to its active substances, in antioxidants or antibacterial activities. These active substances are known as secondary metabolites, classified into phenolics, flavonoids, alkaloids, terpenoids, steroids, and tannins saponin.[4] Tannins are secondary metabolites that belong to polyphenolic compounds commonly present in leafy plants and are water-soluble[5] and classified as condensed tannins (CTs) and hydrolyzable tannins (HTs).[6] They have several benefits, namely as antidiarrheal[7], antibacterial, immunodeficiency of virus (HIV)[8], anti-viral[9], antioxidant, anticarcinogenic, antimutagenic[10], and also bioinsecticide.[11] Tannin analysis is commercially important and has great potential in functional food and pharmaceutical fields. However, the content of phytochemical compounds from *B. pilosa, P. pellucida* L., and *H. corymbosa* L. has not been previously determined by PyGCMS. Therefore, this study aimed to analyze local plants' antioxidant properties and chemical content from Sumenep regency, East Java, Indonesia.

EXPERIMENTAL

Plant Collection and Extraction

Leaves samples (See Table 1) were collected from Sumenep City District, Sumenep regency, East Java, Indonesia. The leave samples were dried for 7 days at room temperature and grinded in a Willey mill to get a dried powder with a mesh size passed to 60 mesh. About 10 g of leave powder was extracted for 3x6 h

with 100 mL of 95% ethanol and aquadest at room temperature. All extracts were conducted for three replicates, and the resulting solvents of extracts were evaporated into a concentrate. The yield of each sample extract was also calculated.

Assessment of Tannins and Phenolic content

A phytochemical test for tannin assessment was conducted for all leave extracts[12]. Further analysis and determination of condensed tannins (CT)[13] and hydrolyzable tannins (HT) were also performed for all leave extracts.[14] Meanwhile, the quantitative analysis of phenolic compound of leave extracts was carried out using a spectrophotometer on the prepared extract and comparison solutions[15]

Assay for Antioxidant Activity

The antioxidant activity of each leaf extract was tested by scavenging of 1,1-Diphenyl-2-Picrylhydrazyl (DPPH).[16] As posit ive control BHT (tert-butylated hydroxytoluene) was used as the positive control. Assays were carried out in triplicate. The IC_{50}, the concentration giving 50% inhibition of DPPH, was read off a graph of I% (percentage inhibition) versus extract concentration.

PyGCMS Analysis

All sample extracts were subjected to pyrolysis-gas chromatography-mass spectrometry (PyGC/MS) according to Ismayati (2016) with temperature profiles of 5 min at 100 °C, 5 min at 50–320 °C (10 °C/min), and 5 min at 320°C.[17]

RESULTS AND DISCUSSION

The crude extract of *B. pilosa, P. pellucida* L., and *H. corymbosa* L. were obtained with ethanol and aquadest as solvents. The yield of the crude extract is presented in Table-1, wherein the ethanol extract shows higher yields than the aqueous extract in all samples. The total yields of ethanol extracts were 20.37% and 19.33% for *P. pellucida* L. and *B. pilosa*. These results showed slightly higher than methanol extract of *P. pellucida* with maceration (10%) and reflux (20%) treatment [18] and ethanol extract of *B. pilosa* about 10.33%.[19] The use of ethanol as solvent extracts were suitable in its application for cosmetic products as an antioxidant agent with bioactive antioxidant compounds.[20]

Table-1: The Yield of Crude Extracts Originated from the Sumenep Regency

Local name plant extractives	Solvent	Code Samples	Yield (%)
			Mean (%)±Stdev
Tongrotong (*Biden pilosa*)	Ethanol (95%)	B-1	19.33 ± 1.53
	Aquadest	B-2	10.6 ± 0.53
Sere Cina *Peperomia pellucida*	Ethanol (95%)	P-1	20.37 ± 0.55
	Aquadest	P-2	12.07 ± 0.60
Lida Ular (*Hedyotis corymbosa*)	Ethanol (95%)	H-1	4.98 ± 0.03
	Aquadest	H-2	2.73 ± 0.25

The presence of natural polyphenols indicated the antioxidant activity[21], and Table 2 shows the phytochemical test for the condensed and hydrolyzable tannin. The antioxidant properties of the plant extracts are strongly influenced by the type of tannin (CT or HT) and the number of hydroxyl units that can reduce the number of free radicals.[22] Table-2 shows the tannin assessment result from plant extract. It was discovered that tannins were present in all the plants as ingredients of the traditional medicine of the Sumenep community. A blackish-green or dark blue color was observed as a reaction plant extracts using $FeCl_3$ solution, whereas tannin formed a complex with Fe3+ ions.[23] Generally, tannins have an O atom in their compound structure, which has a lone pair of electrons acting as ligands to form coordination bonds with Fe3+ ions from $FeCl_3$ as the central atom. The Fe3+ ion is expected to bind three tannins to the O atom

in the 4' and 5'-dihydroxy positions, thereby forming six lone pairs of electrons through coordination bonds. In this position, the O atom has the lowest energy level that allows the formation of complex compounds as ligands. Condensed tannins were found in all plant extracts and hydrolyzable tannins except *B. pilosa* extract (B1 and B2). A hydrolyzable tannin was also not detected in hot and cold water extract from a plant extract of *B. pilosa*.[24] Plant extract of *H. corymbosa* also reported tannin's presence in condensed and hydrolyzable tannins.[25] Further analysis, quantitative tannin was calculated for plant extract samples and described in Table-2. The total percentage of tannin informed that ethanol extract is higher than aquadest extract in all samples. H-1 showed the highest tannin contents, followed by B-1 with values of 153.30% and 112.20%, respectively. B-2, or aquadest extract of *B. pilosa,* has higher than all the aquadest samples of all plant extract. The presence of that type of tannin, CTs, or HTs in plant extracts will affect antioxidant activity

Table-2: The tannin assessment result from plant extracts and quantitative result as a percentage of tannin

Code Samples	Qualitative assay			Phenolic
	Tannin	Condensed tannin (CTs)	Hydrolyzable tannin (HTs)	Mean ppm±SD
B-1	+	+	-	112.20 ± 0.98
B-2	+	+	-	33.57 ± 0.34
P-1	+	+	+	17.98 ± 0.22
P-2	+	+	+	14.55 ± 0.37
H-1	+	+	+	153.30 ± 0.54
H-2	+	+	+	16.70 ± 0.20

Note: (+) is presences and (-) is absences

In this present study, *B. pilosa, P. pellucida* L., and *H. corymbosa* L are tested for antioxidant bioassay using DPPH. The percentage of DPPH reduction results are reported in Table 3. The highest antioxidant properties of plant extracts indicated by the lowest IC_{50} value are 6.43% for B-1, ethanol extract of *B. pilosa*. Furthermore, the lowest IC50 is shown by H-1 and P-1. In other words, ethanol extract's antioxidant properties are higher than aquadest extract's for the same plant extract. These results clearly indicated that the higher tannin content has quantitatively caused the plant extract's higher antioxidant properties. Interestingly, although the H-1 sample has almost the same tannin content as B-1, the IC_{50} value is much higher than B-1. The presence of hydrolyzed tannins may affect the decrease in antioxidant properties of H-1 plant extract sample.

Table-3: Antioxidant activity of leave extracts by DPPH test

Code Samples	Scavenging of DPPH in concentration (ppm), Mean±SD					IC_{50} (ppm)
	10	30	60	90	120	
B-1	26.42±0.3	37.4±0.8	51±0.7	65.07±0.6	69.24±0.6	6.43±0.3
B-2	22.41±1.0	27.82±0.1	38.49±041	50.39±0.3	57.96±0.5	93.61±0.4
P-1	25.04±0.0	37.87±0.2	46.21±0.0	53.94±0.4	64.29±0.6	75.55±0.7
P-2	9.12±0.1	19.47±0.0	29.37±0.1	39.72±0.0	49.61±0.0	119.12±0.0
H-1	23.03±0.8	34.93±0.1	48.22±0.8	57.34±0.9	63.68±0.4	74.44±0.4
H-2	7.57±0.0	19.47±0.0	34.93±0.0	41.89±0.2	48.22±0.6	115.54±0.3

Instead of using GCMS to analyze the extractive content, Py-GCMS was carried out to investigate the pyrolysis products, not only the extractives but also carbohydrates or lignin originating from the plant or lignocellulose biomass[6,26]. At the beginning of retention time, pyrolysis products with low molecular weight originating from acid carbohydrates were detected, such as Pentanoic acid, 3-methyl- (in B1 and B2),

Hexadecanoic acid, Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester; 12,15-Octadecadienoic acid, methyl ester; 6-Octadecenoic acid, methyl ester, (Z)-; 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)- (P1 and P2), Allantoic acid, Propanoic acid, Butanoic acid, methyl ester, Butanoic acid, 2-Amino-3-methyl-4-pentynoic acid (in H-1 and H-2).

Phenol, 2-methoxy- or guaiacol (G) was also detected as lignin (G-unit) derivatives in B1 and B2, while 2-Methoxy-4-vinyl phenol was detected in H-1 and H-2. The guaiacol pyrolysis product also can be originated from polyphenol (tannin or flavonoid)[6] or lignocellulose biomass.[27] The presence of lignin G-units may come from carbohydrates in which the pyrolysis process separated conjugate to lignin as secondary metabolites.[22] Malvindin-3-O-glucoside (anthocyanin) is a polyphenolic compound conjugated with carbohydrates and was detected as malvindin and 3-O-glucoside as a pyrolysis product.[28] Polyphenol and lignin have antioxidant activity[29], but the impurities of carbohydrates that are soluble in aquadest plant extracts might be reduced the antioxidant activity.[30]

Based on the PyGCMS result (Table 4-6), the pyrolysis product from plant extract was identified as terpenoid, steroid, and tannin or flavonoid. The B-1 has 9,12,15-Octadecatrienoic acid (Z, Z, Z)-as major with a relative abundance of about 26.26%, respectively, with antioxidant, antimicrobial, and anticancer properties reported in the previous study.[31] Moreover Neophytadiene, Phytol, squalene, gamma.-Tocopherol (terpenoid) and Stigmasta-5,22-dien-3-ol, acetate, (3.beta.,22Z)- (steroid) were detected. These chemical compounds have bioactivities such as antimicrobial.[32,33] gamma.-Tocopherol was reported as an antioxidant agent in food products.[33] The presence of terpenoid of *B. pilosa* was supported by the founding stigmasterol, squalene in leave extract.[34,35,36] In P-1 and P-2 (*P. pellucida)*, the major components shown by Lup-20(29)-en-3-ol, acetate, (3.beta.)- and 6-Octadecenoic acid, methyl ester, (Z)- with total relative abundance about 25.81% and 23.22%, respectively. Another terpenoid (Phytol, beta.-Amyrin) was also detected in P-1 and P-2. Furthermore, Braleyin and rupeol were detected in *H. corymbosa* L (H-1 and H-2) at low relative abundance. Based on this study, the three plants of Sumenep medicinal plants are potential sources of polyphenols for food, medicine, and supplement and have potential in the manufacturing industries.

Table-4: Pyrolysis products of Tongrotong (*Biden pilosa*) sample extract analyzed by PyGCMS

No	Rt (min)	Pyrolysis product	Relative abundance (%)	
			B1 (Ethanol (95%)	B2 (Aquadest)
1	2.668	Pentanoic acid, 3-methyl-	3.44	22.92
2	3.095	Pentanoic acid, 3-methyl-	N.d	9.65
3	3.208	(R)-1,4-Diformyloxy-2-cyanobutane	N.d	14.44
4	4.161	Toluene	N.d	3.78
5	5.772	1,2-Cyclohexanedione	N.d	2.48
6	7.542	Phenol, 2-methoxy-	1.54	4.14
7	10.484	Benzofuran, 2,3-dihydro-	4.64	N.d
8	12.021	2-Methoxy-4-vinyl phenol	1.77	6.35
9	12.705	Eugenol	3.39	N.d
10	13.788	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decane-rel-	1.11	N.d
11	13.982	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decane-rel-	0.7	N.d
12	14.729	1-Methylcyclohexylcarboxylic acid	N.d	12.04
13	17.765	Acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester	1.13	N.d
14	18.156	Benzene, 1,3,5-heptatriyn-1-yl-	1.46	N.d
15	19.084	Neophytadiene	13.2	1.69

16	19.546	Neophytadiene	5.08	N.d
17	20.044	Hexadecanoic acid, methyl ester	3.13	2.82
18	20.648	n-Hexadecanoic acid	2.85	2.5
19	21.835	10,13-Octadecadienoic acid, methyl ester	1.4	8.35
20	21.927	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	6.75	N.d
21	22.058	Phytol	6.8	2.38
22	22.673	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	26.26	4.72
23	28.188	Squalene	8.61	1.74
24	30.72	.gamma.-Tocopherol	3.7	N.d
25	30.868	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.,22Z)-	3.04	N.d

Note: N.d means not detected

Table-5: Pyrolysis products of Sere Cina (*Peperomia pellucida*) sample extract analyzed by PyGCMS

No	Rt (min)	Pyrolysis product	Relative abundance (%)	
			P1 (Ethanol (95%)	P2 (Aquadest)
1	15.644	1,2-Dimethoxy-4-(2-methoxyethenyl)benzene		2.77
2	16.497	Carotol		6.07
3	16.567	Apiol		7.13
4	16.801	Isospathulenol		1.39
5	19.013	Neophytadiene		1.55
6	19.499	Neophytadiene		0.9
7	19.889	9-Hexadecenoic acid, methyl ester, (Z)-	7.94	
8	20.025	Hexadecanoic acid, methyl ester		14.68
9	20.075	Hexadecanoic acid, methyl ester	20.16	4.07
10	21.029	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	2.25	
11	21.836	12,15-Octadecadienoic acid, methyl ester		16.93
12	21.981	6-Octadecenoic acid, methyl ester, (Z)-	23.22	
13	21.922	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-		31.73
14	22.024	Phytol, beta.-Amyrin		3.43
15	22.151	Methyl stearate	3.38	3.11
16	22.416	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-		3.24
17	27.78	.beta.-Amyrin	8.46	
18	27.966	Dibenzo[c,l]chrysene-8-carboxylic acid, methyl ester		3.01
19	28.866	Lup-20(29)-en-3-ol, acetate, (3.beta.)- and 6-Octadecenoic acid, methyl ester, (Z)-	25.81	
20	30.342	Friedelan-3-one	8.77	

Note: N.d means not detected

Table-6: Pyrolysis products of Lida Ular (*Hedyotis corymbosa*) sample extract analyzed by PyGCMS

No	Rt (min)	Pyrolysis product	Relative abundance (%)

			H1 (Ethanol (95%)	H2 (Aquadest)
1	2.54	1-Ethyl-1-methylhydrazine	N.d	6.32
2	2.602	Allantoic acid	N.d	9.56
3	2.79	Propanoic acid	N.d	4.67
4	2.879	Butanoic acid, methyl ester	N.d	3.9
5	3	2-Butanol, 1-benzyloxy-3-methyl-	N.d	2.04
6	3.198	Butanoic acid	N.d	13.14
7	3.579	2-Amino-3-methyl-4-pentynoic acid	N.d	0.99
8	7.444	Phenol, 2-methoxy-	N.d	2.64
9	7.888	Benzoic acid, methyl ester	2.62	N.d
10	11.401	trans-4-Hydroxycyclohexanecarboxylic acid, methyl ester	N.d	1.53
11	11.939	2-Methoxy-4-vinyl phenol	N.d	1.11
12	19.079	Neophytadiene	1.1	N.d
13	20.052	Hexadecanoic acid, methyl ester	16.98	N.d
14	20.544	n-Hexadecanoic acid	5.58	N.d
15	21.824	12,15-Octadecadienoic acid, methyl ester	14.47	N.d
16	21.895	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	21.58	N.d
17	22.041	Phytol	3.48	N.d
18	22.101	Methyl stearate	5.69	N.d
19	22.33	cis-9-Tetradecen-1-ol	2.63	N.d
20	22.399	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	3.48	4.36
21	22.94	Stigmasterol	N.d	7.34
22	23.02	Stigmasterol	N.d	5.22
23	24.509	Brayelin	3.24	N.d
24	24.584	9-Octadecenamide, (Z)-	4.83	N.d
25	24.985	.gamma.-Sitosterol	N.d	3.22
26	25.05	.gamma.-Sitosterol	N.d	1.26
27	25.745	Docosanoic acid, methyl ester	1.4	N.d
28	25.889	Bis(2-ethylhexyl) phthalate	8	N.d
29	26.579	Lup-20(29)-en-3-one	N.d	2.56
30	27.26	Lupeol	N.d	4.53
31	27.92	Olean-12-en-3-ol, acetate, (3.beta.)-	N.d	3.69
32	28.132	Squalene	4.92	N.d
33	29.01	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	N.d	15.55
34	29.23	Phytyl stearate	N.d	1.61
35	29.966	D:A-Friedooleanan-3-ol, (3.alpha.)-	N.d	2.72
36	30.505	Friedelan-3-one	N.d	7.24

Note: N.d means not detected

CONCLUSION

The leaf extract of *B. pilosa*, *H. corymbosa*, and *P. pellucida* showed potency as an antioxidant. The highest antioxidant properties were presented by B-1 with an IC_{50} value of about 6.43% with total phenolic content of 112.20 ppm. The presence of carbohydrate derivatives in H-1 decreased the antioxidant properties (IC_{50} value and Phenolic contents are 74.44% and 153.30ppm). The phenolic derivatives products detected by

PyGCMS were tannin, steroid, terpenoid, and steroid. Based on the results, the B-1 extract has the potential as an antioxidant additive in the food, cosmetic, or advanced materials industry.

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