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Bioactive compound and Antioxidant Activity Analysis of Some Medicinal Plants of Province of Western Sulawesi

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Abstract: The main objective of this study was the screening of some selected medicinal plants very popular in Provinsi of western Sulawesi, with respect to antioxidant capacity, and bioactive compound. All plants were extracted with the conventional method, maceration with methanol. Bioactive compound was measured by GCMS-QP2010 Ultra Shimadzu. The antioxidant capacity of the plant extracts was measured by their ability to scavenge free radicals such as DPPH (2,2-diphenyl-1-picrylhydrazyl). These extracts resulted in a rapid increase and decrease of the absorbance and showed different hydrogen-donating capacity towards the DPPH radical. A lot of differences found and showing anti-oxidant activity of methanol extracts of different plant species. Among the species, methanol extract of *Ficus septica*, *Cordoline sp.*, *Celotia argantea*, *Physalis angulate*, *Kalandioe pinnata* and *Melostoma polyanthum*, showed the maximum scavenging capacity of over 70.

1. Introduction

Indonesia has been known for its greatest biodiversity in the world, comprising tropical plants and marine biota. In the Indonesian territory there are about 30,000 species of plants and 7,000 of them are thought to have medicinal properties. One province in Indonesia is the province of western Sulawesi, also has several types of medicinal plants that can be used in the treatment of various types of diseases. Diseases that can be cured include, diabetes mellitus, hypertension, diarrhea, tetanus, anemia, and others [1].

Medicinal plants have different properties. The efficacy of medicinal plants is highly dependent on the bioactive components contained therein. With the bioactive components, the medicinal plants are able to neutralize the cause of a disease. Based on the potential of medicinal plants in West Sulawesi, the analysis of bioactive components and antioxidant activity on the types of medicinal plants commonly used by local communities. It is expected from this research to know what bioactive components. In this study, we investigated 6 selected, local putative medicinal plants for their potential antioxidant activities using scavenging free radical activity assays [2].

The aim of this study is to determine the organic compounds present in the medicinal plant extract with the aid of GC-MS technique, which may provide an insight in its use in tradition medicine.

2. Plant material

Leaves of medicinal plant were selected to screen its biopotentials based on its traditional usage. Care was taken to select healthy leaf. The plant were cut into small pieces and shade dried at room temperature for 15 days. Preparation of extracts medicine plants the collected was washed under running tap water and dust was removed from the leaves. The leaves were dried at room temperature



for 15 days and coarsely powdered. The powder (2 gm) was extracted with 70% methanol and 100% aqueous for 48 hours. A semi solid extract was obtained after complete elimination of alcohol and water under reduced pressure. The extract medicine plants was stored in refrigerator until used. Chemical tests were carried out on the alcoholic and aqueous extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Sofowara, Trease and Evans and Harborne.

Preparation of extracts medicine plant the above said herbs were selected and procured from the approved supplier. They were washed with water and then powdered. The powder was taken and extraction was carried out in large scale capacity reactor using 75% methanol and concentrated. The concentrated extract was spray dried and the dried powder was taken to check the antioxidant activity and GC-MS analysis.

2.1. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis analysis were carried out on a GC-MS-QP 2010 Plus Shimadzu system and Gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column (30m x 0.25mm ID x μ l df, composed of 100% dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2 μ l was employed (Split ratio of 10:1) injector temperature-250oC; ion-source temperature 280oC. The oven temperature was programmed from 110oC (Isothermal for 2 min.) with an increase of 10oC / min to 200oC then 5oC / min. to 280°C / min, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5s and fragments from 40 to 550Da. Total GC running time was 36 minutes. The relative percentage amount of each component was calculated, by comparing its average peak area to the total areas, Software adopted to handle mass spectra and chromatogram was a turbomass. The detection employed the NIST Ver. 2.0 year 2009 library [3].

2.2. Free radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay DPPH radical scavenging activity was done using the reported method; the reaction mixture containing 1 mL of DPPH solution (0.1 mmol /L, in 95% methanol v/v) with different concentrations of the extract was shaken and incubated for 20 min at room temperature and the absorbance was read at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and calculated using the following equation: Effect of scavenging (%) = $[1 - A_{\text{sample}}(517\text{nm}) / A_{\text{control}}(517\text{nm})] \times 100$

3. Result and Discussion

Extracts were subjected for the evaluation of antioxidant activity by using various in vitro model systems. DPPH radical scavenging activity was observed in all the extracts, the curcuma extract showed dominant activity followed by coffee bean extract among the extracts. The IC50 values were calculated and are depicted in (Table 1).

Table 1. DPPH dengan persentase penghambatan dan kapasitas antioksidan dalam meredaksi radikal bebas DPPH 0.1 mM. Each values represents SD \pm mean (n=3)

Sample	% of DPPH Scavenging activity	Antioxidant capacity (mg/L)
<i>Ficus septica</i>	61.186 \pm 0.514	60.679 \pm 0.249
<i>Cordolone sp</i>	32.995 \pm 0.724	38.598 \pm 0.2746
<i>Celotia argantea</i>	36.837 \pm 0.570	43.707 \pm 0.260
<i>Physalis angulate</i>	41.136 \pm 0.837	41.216 \pm 0.277
<i>Kalandioe pinnata</i>	92.065 \pm 0.365	79.586 \pm 0.415
<i>Melostoma polyanthum</i>	94.617 \pm 0.207	80.797 \pm 0.155

Table 1 shows that *polyantuhum melostoma* plant has a percentage of DPPH scavenger activity ($94.617 \pm 0.207\%$) and antioxidant capacity ($80.797 \pm 0.155 \text{ mg / l}$) is highest. Further follow-up plants that have DPPH scavenging activity are *Kalandioe pinnata*, *Ficus septica*, *Physalis angulate*, *Celotio argantea* and *Cordoline sp.* And for plants that have antioxidant capacity are *Kalandioe pinnata*, *Ficus septica*, *Celotio argantea*, *Physalis angulate*, and *Cordoline sp.*

The results of the GC-MS analysis of the methanolic extract of *Kalandioe pinnata*, *Ficus septica*, *Celotio argantea*, *Physalis angulate*, *Cordoline sp* and *Melostoma polyantuhum* are given in Table 1. The major components were 2,3,5,6-tetramethyl-benzenesulfonamide (0.65), Tridecane, 6-methyl (3.33), Tetradecane (3.56), Hexadecane (2.37), Octadecane (1.46), Phthalic acid, butyl hept-2-yl ester (5.27), Phytol (2.94), 9Cyclohexylnonadecane (15.93), 9-Octadecenamide, (Z) (18.89), 2,2,2-trifluoroethyl 2-methyltetrahydro-5-oxo-3furanocarboxylate(10.47) (Table 2)

Table 2. The chemical composition of methanolic extract of *Kalandioe pinnata*, *Ficus septica*, *Celotio argantea*, *physalis angulate*, *Cordoline sp* and *Melostoma polyantuhum*

1 <i>Ficus septica</i>				
Peak	R. Time	Name	Molecular weight	Formula
50	20.674	Phytol	296	C20H40O
38	16.840	2-hexadecen-1-ol, 3,7,11,15-tetramethyl	296	C20H40O
19				
62	12.946	1,3-Propanediol, (hydroxymethyl)-2-nitro	2- 151	C4H9NO5
41	17.406	2-hexadecen-1-ol, 3,7,11,15-tetramethyl	296	C20H40O
44	18.002	hexadecanoic acid, methyl ester	256	C16H32O2
30	15.089	3-Buten-2-ol, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)	197	C13H22O
36	37.092	Vitamin E	430	C29H50O2
67	35.328	gamma.-Tocopherol	417	C28H48O2
2 <i>Cordoline sp</i>				
Peak	R. Time	Name	Molecular weight	Formula
47	35.184	lup-20(29)-en-3-yl acetate	468	C32H52O2
39	26.790	hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	330	C19H38O4
39	18.003	hexadecanoic acid, methyl ester	270	C17H34O2
44	37.090	Vitamin E	430	C29H50O2
31	10.126	2-Methoxy-4-vinylphenol	150	C9H10O2
3 <i>Celocasia argantea</i>				
Peak	R. Time	Name	Molecular weight	Formula
22	16.638	2-hexadecen-1-ol, 3,7,11,15-tetramethyl	296	C20H40O
29	18.147	hexadecanoic acid, methyl ester	270	C17H34O2
34	20.668	Phytol	296	C20H40O
25	17.404	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-	296	C20H40O
33	35.325	gamma.-Tocopherol	416	:C28H48O2
9	37.090	Vitamin E	430	C29H50O2

4 <i>Physalis angulate</i>				
Peak	R. Time	Name	Molecular weight	Formula
11	6.325	diisodecyl ether	298	C20H42O
12	6.386	1-decene, 2,4-dimethyl	168	C12H24
29	9.858	1-tridecanol	200	C13H28O
31	5.145	decane, 2,3,4-trimethyl	184	C13H28
9	37.089	Vitamin E	430	C29H50O2
5 <i>Kalanchoe pinnata</i>				
Peak	R. Time	Name	Molecular weight	Formula
14	20.509	9-Octadecenoic acid (Z)-, methyl ester	296	C19H36O2
12	18.003	Hexadecanoic acid, methyl ester	270	C17H34O2
17	20.887	Octadecanoic acid, methyl ester	298	C19H38O2
13	20.409	9,12-Octadecadienoic acid, methyl ester	294	C19H34O2
16	20.668	Phytol	296	C20H40O
7	15.267	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	194	C11H14O3
6 <i>Melostoma polyanthum</i>				
Peak	R. Time	Name	Molecular weight	Formula
41	24.671	Delta.-1(2)-tetrahydrocannabinol	251	C15H25NO2
24	20.664	Phytol	336	C18H18B2O5
43	25.916	Dibenz[d,f]cycloheptanone, 2,3,9-trimethoxy	288	C20H32O
49	27.993	1,4-phenanthrenedione, 3-(acetyloxy)-4b,5,6,7,8,8a,9,10-octahydro	344	C21H28O4
18	18.531	n-Hexadecanoic acid	296	C20H40O

The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in Table 2. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. The GC-MS study of the methanolic extract of The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in Figure1. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. The GC-MS study of the methanolic extract of the plants had shown the presence of lots of phytochemicals which strength contribute to the medicinal bioactive of that plant had shown the presence of lots of phytochemicals which strength contribute to the medicinal bioactive of that plant.

The identified major compounds possess some important biological potential for future drug development. There is growing awareness in correlating the phytochemical compounds and their biological activities. The identified major compounds possess some important biological potential for future drug development. There is growing awareness in correlating the phytochemical compounds and their biological activities. Similar to this study, five major compounds were characterized through GC-MS analysis in Polygonum chinense [4]. Eighteen phytochemical constituents have been

identified from the ethanolic extract of the leaves of *Desmodium gyrans* by Gas chromatogram Mass spectrometry (GC-MS) [5]. Nanadagopalan reported the presence of Phytol in the leaves of *Kirganelia reticulata* aerial parts, which was found to be effective in different stages of arthritis [6]. 9-octadecenoic acid (20.89%) constitutes the major constituent of the leaf extract while oleic acid (84%) is the major component of the seed extract [7]. In spite of the advantage of modern high drug discovery and screening techniques, traditional medicinal knowledge have also given clues to the discovery of valuable drugs [8]. There is growing awareness in correlating the phytochemical compounds with their biological activities. GC-MS analysis of ethanol extract has led to identification of twenty-eight compounds from *Macrotyloma uniflorum* Linn. by comparison of their retention indices and mass spectra fragmentation [9]. The ethanolic leaf extract obtained from *P. pulchellum* were subjected to chemical analysis by GC-MS method which confirmed the presence of phytochemicals which are responsible for pharmacological activities

4. Conclusion

The presence of bioactive compounds justifies the use of the leaf part for various ailments by traditional practitioners. The present study aimed at identifying the nature of the components responsible for their antioxidant activity. This study clearly shows that GC-MS is a powerful technique enabling fast separation and characterization of bioactive metabolites. The high sensitivity of this technique helps in characterization of active compounds in *Kalandioe pinnata*, *Ficus septica*, *Celotia argentea*, *Physalis angulate*, *Cordolone sp* and *Melostoma polyanthum*. In addition to the antioxidant activity test showed Among the species, methanol extract of *Ficus septica*, *Cordolone sp.*, *Celotia argentea*, *Physalis angulate*, *Kalandioe pinnata* and *Melostoma polyanthum*, showed the maximum scavenging capacity of over 70.

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