Characterization of the chemical composition of *Adenostemma lavenia* (L.) Kuntze and *Adenostemma platyphyllum* Cass

A Fauzan[1, 2], D Praseptiangga[2, 3], R Hartanto[2, 3] and B Pujiasmanto[2, 4]

1. Department of Agrotechnology, Faculty of Agriculture, Universitas Pekalongan, Jl. Sriwijaya No. 3 Pekalongan, Indonesia
2. Graduate School Program of Universitas Sebelas Maret, Jl. Ir. Sutami 36 A, Kentingan, Surakarta 57126, Indonesia
3. Department of Food Science and Technology, Faculty of Agriculture, Universitas Sebelas Maret, Jl. Ir. Sutami 36 A, Kentingan, Surakarta 57126, Surakarta, Indonesia
4. Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Jl. Ir. Sutami 36 A, Kentingan, Surakarta 57126, Surakarta, Indonesia

e-mail : zan.anwar@gmail.com

Abstract. The purpose of this study was to characterize the chemical compounds of *Adenostemma lavenia* (L.) Kuntze (Al) and *Adenostemma platyphyllum* Cass (Ap) using Pyrolysis-gas chromatography/mass spectrometry (Py-GCMS) and proximate analysis. Two species of *Adenostemma* samples (roots, stem and leaves) about 1 mg was pyrolyzed directly at the optimum temperature of 600°C. Py-GCMS was relatively fast, easy to use and without samples preparation and identification of the chemical compounds was carried out by comparison of the mass spectra obtained with those stored in Wiley 7th libraries. The data of proximate analysis were statistically analysed using Friedman test followed and hierarchical cluster analysis (HCA) for data of Py-GCMS. The result of proximate analysis showed that *A. lavenia* (L.) Kuntze (Al) and *A. platyphyllum* Cass (Ap) contained 8.27% (*Al*) and 9.18% (*Ap*) of water, 11.52% (*Al*) and 17.84% (*Ap*) of protein, 5.67% (*Al*) and 6.33% (*Ap*) of fat, and 17.32% (*Al*) and 19.94 (*Ap*) of ash. Amines, aldehydes, fatty acids, terpenoids-steroids, alkaloids, aromatic and aliphatic hydrocarbons, phenolic, and oligopeptides as part of 125 chemical compounds of each species are identified by Py-GCMS analysis. Hierarchical cluster analysis of pyrolysis products indicate not similitary of major chemical compounds of two *Adenostemma* species.

Keywords : Adenostemma lavenia, Adenostemma platyphyllum, Py-GCMS, Friedman test, . hierarchical cluster analysis

1. Introduction

The study of *Adenostemma* biology and ecology has been receiving growing attention worldwide [1-7], but study of identification basic ecological relationships and chemical compounds is low. Recent studies have found 11-hydroxilated kauranic acids [8], kaurane [9, 10], α-*cubebene*, *caryophyllene*[10], flavanoids and alkaloids [11]. The chemical composition of *Adenostemma* differs depending on species, growth environment and season [12-16, 18], age and plant parts [17], physiological variations, environmental conditions, geographic variations, genetic factors and evolution [19].

So far, several methods have been developed to analyze the chemical compounds of *Adenostemma*. Phytochemical analysis [11], spectrographic [7, 8], high performance liquid chromatography (HPLC) [8, 9] were used to investigate the chemical compounds of *Adenostemma*. However, troublesome pretreatments are necessary for all the above methods prior to the final analysis such as solvent extraction, purification, and derivatization. Most of the studies were limited to some components of interest, particularly the flavonoids, lacking of a whole chemical composition analysis. Therefore, it is meaningful to develop a simple, rapid and sensitive method for characterization of the whole chemical composition in *Adenostemma*.

Recently, py–GCMS has been used extensively in the characterization of both low and high molecular components in various natural products. This technique yields a pyrogram consisting of the characteristic peaks of constituents in the given natural product without any pretreatment. Py–GCMS has been

performed for accurate determination of terpenoids, alcohols in volatile constituents in *Houttuynia cordata* Thunb [16], phenolic compounds in origanum heracleoticum [17], and terpenic acids, aleuritic acid, fatty acids in natural resin shellac [18]. Organic compounds in plants are divided into two main groups, Nitrogen-containing (alkaloids, non-protein amino acids, amines, cyanogenic, glycosides, glucosinolates, alkamides, lectins, peptides, polypeptides) and without nitrogen (terpenoids, flavonoids, tannins, phenylpropanoids, lignin, coumarins, lignans, Polyacetylenes, fatty acids, waxes, Polyketides, Carbohydrates, organic acids) [20].

In this study, Py–GCMS was first applied to analysis of chemical composition in *Adenostemma* without any cumbersome pretreatment. Furthermore, two species samples obtained from The northern slope of Dieng plateau were also compared, based on the chemical compound of *Adenostemma*. The aim of this paper is to analyze and compare the chemical compositions of two *Adenostemma* species using destructive analytical methods.

2. Material and Method

2.1 *Adenostemma sampling*

Fresh *Adenostemma* material from two species was collected in the growing season of 2016 in north slope Dieng plateau (07°3'20.29" - 7°10'25.09" N; 109°40'21.33" – 109°36'20,09" E; 200 – 1,000 m amsl) in tropical rain forest (Java, Indonesia). Only living material with green leaves and fresh flower without signs of decomposition was collected. Samples were identified at Center for Plant Conservation Botanic Garden – Indonesian Institute of Science as *Adenostemma platyphyllum* Cass. and *Adenostemma lavenia* (L.) Kuntze.

2.2 *Sample preparation*

The approximate amount of collected adenostemma material was 50 g dry weight species. The samples was cleaned. The roots, stems and leaves were cut out from some plants; others were kept intact. Afterwards, the roots (Ap1 and Al1), stems (Ap2 and Al2), leaves (Ap3 and Al3) and entire plants (Ap and Al) were oven-dried separately at 40ºC until dry weight was constant and were stored at 5ºC in air-tight containers before analysis. Samples for proximate analysis and Py-GCMS analysis were dried, ground with a grinder and sieved through a 40 mesh [21].

2.3 *Proximate Analysis*

The proximate analysis (water, proteins, crude fats, ash, carbohydrates and total dietary fiber) of all the samples carried out in triplicate [22]. The water and ash were determined using gravimetric method. The nitrogen value was determined by micro-Kjeldahl method described by [23] involving digestions, distillation and finally titration of the sample. The nitrogen value was converted to protein by multiplying a factor of 6.25. The crude fat was determined by soxhlet extraction method, employing a n-hexane extraction. Carbohydrate was determined by difference method. Carbohydrate was determined when the sum of the percentages of water, ash, crude protein, and crude fat were subtracted from 100. All the proximate values were reported in percentage [22].

2.4 *The Pyrolysis GCMS Analysis*

The chemical compounds in sample were identified by a Pyrolysis GCMS (Shimadzu GCMS-QP2010; Shimadzu Corporation, Kyoto, Japan). One milligram ±0.05 mg of powdered sample was introduced into a pyrolyzer (PY-2020iS). Pyrolysis was performed at a temperature of 600°C with a ramp rate of 20°C/ms with a hold time of 10 s. The pyrolyzer was directly interfaced with a GCMS. A capillary column (RTX-5MS) with 60 m length, 0.25 mm internal diameter and a 0.25 μm stationary phase film was used. The volatiles were trapped on adsorbent trap before being desorbed at 280 °C onto a heated transfer line which was held at 280°C. The purge flow of helium UHP to remove any oxygen from the sample, prior to pyrolysis, was set to 3 ml/min and a split ratio 1:50. The injector temperature was set at 280ºC, ion source 200ºC. The identification of the chemical compounds was carried out by comparison of the mass spectra obtained with those stored in Wiley 7th libraries [24].

2.5 *Data Analysis*

The data of proximate analysis were statistically analysed using Friedman test followed with Post Hoc test using Wilcoxon Signed Rank test, and hierarchical cluster analysis (HCA) was used to identify relatively homogeneous clusters of samples based on their similarity [25] in chemical compound Adenostemma's. Cluster analysis is technique for grouping object into clusters so that object in same cluster. A cluster are more like one another than they are like object in other cluster [26]. Cluster analysis involves at least two step. The first is the measurement of some form of similarity to determine how many groups really exist in the sample [26, 27]. The second step is to profile the variable to determine their composition [28]. Beginning with N clusters consisting exactly of two entities, the similarity matrix is searched for the most

similar pair of clusters and the number of clusters is reduced by one by merging the most similar pair of clusters with the minimum increase in the total within group error sum of squares [21]. The analysis was performed using IBM SPSS Statistics 23 version software.

Table 1. List of studied *Adenostemma* species with codes and their growth conditions

Species	Code	Growth conditions
A. platyphyllum	Ap	Tropical rainforest with soil pH 6.9 (± 0.93); soil carbon 1.32% (±0.15); total organic matter 2.28% (±0.26); nitrogen 0.25% (± 0.07); available phosphorus 4.38 ppm (± 0.83); K (me) 0.27 (± 0.03); Ca 1.74 mg (±0.49); Mg 1.30 (±0.57); C/N ratio 5.28 (± 0.48); latosol; 591 m amsl; the average max. and min. temperatures are 26.81°C and 18.78°C; the average annual rainfall is 55.91 cm; RH 93%. Ecological indicator [29]: Diversity (b) 3.164; Dominance (Cd) 0.177, Evenness Index (J') 0.8446; Richness Index (al) 23; Shannon-Wiener Index (H') 2.648
roots	Ap1	
stems	Ap2	
leaves	Ap3	
A. lavenia	Al	Tropical rainforest with soil pH 6.8 (± 0.85); soil carbon 1.33% (±0.07); total organic matter 2.30% (± 0.12); nitrogen 0.26% (± 0.04); available phosphorus 4.8 ppm (± 2.16); K (me) 0.31 (± 0.09); Ca 1.20 mg (± 0.69); Mg 0.60 (± 0.03);C/N ratio 5.12 (± 0.55); latosol; 930 m amsl; the average max. and min. temperatures are 22.06°C and 15.5°C; the average annual rainfall is 60.80 cm; RH 95%. Diversity index of herbs [29]: Diversity (b) 3.183; Dominance (Cd) 0.193, Evenness Index (J') 0.85088; Richness Index(al) 24; Shannon-Wiener Index (H') 2.704
roots	Al1	
stems	Al2	
leaves	Al3	

3. Results and discussion

3.1 *Proximate analysis*

The result of proximate analysis shows variant concentration of chemical compounds (water, protein, fat, ash, carbohydrate and total dietary fiber). Based on Friedman test analysis, the water contents of samples are different. The water contents of whole *A platyphyllum* were higher than *A lavenia*. Considering the overall percentage of water composition, it was highest in *A platyphyllum* stems followed by *Aplatyphyllum* roots, *Aplatyphyllum* leaves and *A lavenia* leaves while other had comparatively lesser composition (Table 2). However, the water in dry powder of stems and roots of *A lavenia* are the highest.

Table 2. Proximate analysis of *Adeostemma* sp.

Code	Water		Protein*	Fat*	Ash*	Carbohydrate*	Fiber, total dietary*
	Fresh	Powder					
Ap	89.54 b	9.18 d	17.84 f	6.33 d	19.94	55.89 b	2.41 b
Ap1	90.66 d	7.45 b	8.85 a	3.61 b	16.47	71.07 d	6.27 f
Ap2	91.73 e	9.39 f	12.49 c	7.31 f	21.24	58.96 c	2.36 a
Ap3	89.64 c	7.17 a	18.38 g	10.50 g	21.00	50.12 a	2.62 c
Al	88.56 a	8.27 c	11.52 e	5.67 c	17.32	68.41 c	2.62 c
Al1	89.35 b	9.58 g	11.52 b	6.71 e	15.05	67.64 d	6.75 g
Al2	90.61 c	10.36 h	10.60 b	2.75 a	17.32	68.41 c	3.40 d
Al3	88.82 a	9.34 e	13.37 d	10.93 h	16.92	58.79 d	3.72 e
Friedman test							
Chi-Square	15.667	21.000	18.556	21.000	11.194	19.104	20.556
Df	7	7	7	7	7	7	7
Asymp. Sig	0.028^{s}	0.004^{s}	0.010^{s}	0.004^{s}	0.130^{ns}	0.008^{s}	0.004^{s}

Note: Data are means from three samples. * : dry basis; ns: non-significant, s: significant at P 0.05 Friedman test. Figures followed by the same letter in the same column are not significantly different at 5% level Wilcoxon Signed Rank test.

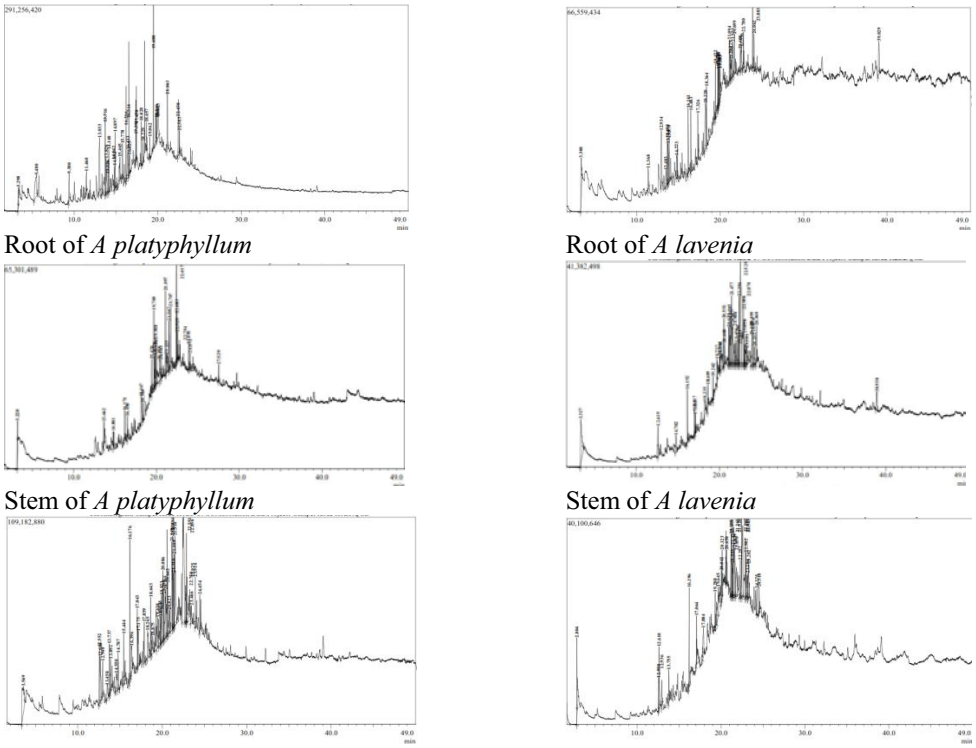
The protein contents of each species and organs are different. Considering the overall percentage of protein composition, it was highest in *A. platypyllum* leaves by whole of *A. platypyllum*. Whole of *A. lavenia*, and *A. lavenia* leaves while other had comparatively lesser composition (Table 2). Considering the result crude fat. *A. lavenia* leaves and *A. platyphyllum* leaves had prominent levels compared to other plant organs. Considering the resulted achieved from ash analysis. The ash contents of each species are not different. While analyzing the carbohydrate contents in the samples. The results showed that *A platyphyllum roots*. Whole of *A. lavenia*. *A. lavenia* roots and *A.lavenia* stems had highest concentration of carbohydrate to other plant organs (Table 2).

Based on the proximate analysis it is known that two species of *Adenostemma* exhibit different characteristics, except ash content.Different concentrations of chemical compounds are caused by differences in species. plant organs and growing places of each samples [20]. The sample of this study was

obtained from wild forests that have different ecology, macro and microclimates (temperature, water, humidity, sunlight intensity)..

3.2 *Pyrolysis GCMS Analysis*

Py-GCMS is a suitable method for the quantitative and qualitative analysis of complex mixtures with high efficiency, precision, and simplicity [30, 31]. In a merbau extractives pyrolysis study, flash pyrolysis-GCMS used in structure analysis provided information at the presence of phenolic forms [24]. Pyrolysis-gas chromatography/mass spectrometry was used to characterize the chemical composition of *A. platyphyllum* and *A lavenia*. The result of Py-GCMS analysis to those sample found 125 chemical compounds. Among all, the 5 compounds dominant were: epoxycyclododecane, 4-allyl-2,6-dimethoxyphenol, Cis,Cis,Cis-8,11,14 eicosatrienoic acid, tetradecahydroanthracene, and levoglucosan. The chemical compounds in the studied two species *Adenostemma* can be seen in Appendix 1. GCMS analysis revealed the presence of terpenoids, phenolic compounds, alkaloids and fatty acids.



Root of *A platyphyllum* Root of *A lavenia*

Stem of *A platyphyllum* Stem of *A lavenia*

Leaves of *A platyphyllum* Leaves of *A lavenia*

Fig. 1. Chromatogram py-GCMS of *Adenostemma platyphyllum* and *Adenostemma lavenia*

The presence of dominant product groups from each *Adenostemma* species is illustrated in Table 3. The pyrolysates of the studied *Adenostemma* species were dominated by phenolic compounds, lipid orginated compounds and N-bearing compounds. The dominant compounds in *A. platyphyllum* were phenolic compounds and originated lipids (fatty acid). The dominant compounds in *A. lavenia* were N-bearing compounds, alkaloid, aromatic compounds, and terpenoid-steroid. Abundant aromatic compounds products (phenol and derivate) confirm the importance of phenol in the structural make-up of *Adenostemma*. As one of the main products. *A lavenia* species contained more terpenoid-steroid and alkaloid in comparison with *A. platyphyllum* species. *A lavenia* species has a higher alkaloid compounds than *A. platyphyllum*.

4-Allyl-2,6-Dimethoxyphenol ($C_{11}H_{14}O_3$), coniferyl alcohol ($C_{10}H_{12}O_3$), and linoleic acid ($C_{18}H_{32}O_2$) were the dominant phenolics found in *Adenostemma* species. 4-Allyl-2,6-Dimethoxyphenol was phenol detected in the largest quantity (14.2%). 4-O-[3-Acetyl-1-(Trimethylsilyl)-1h-Indolyl]-D-Glucose ($C_{32}H_{62}N_2O_7Si_5$; 2.14%) and 5h-1-Pyrindine (C_8H_7N; 5.07%)were the alkaloid found in the leaf tissue of *A lavenia*. 3-methylindole (C_9H_9N; 0.93%) and 1-cyano-3-methylisoquinoline ($C_{11}H_8N_2$; 1.83%) were the alkaloid found in the stem tissue of *A lavenia*. 6,7-Dihydro-3-Nitro-5h-Cyclopenta[B]Pyridin-2(1h)-One ($C_8H_8N_2O_7$; 2.94%) and 5,10-Diethoxy-2,3,7,8-Tetrahydro-1h,6h-Dipyrrolo[1,2-A;1',2'-D] Pyrazine ($C_{14}H_{22}N_2O_2$; 4.0%) were found in the root tissue of *A lavenia*. The alkaloids were detected in *A platyphyllum* only two substance i.e. 3-Methylindole (1.62%) and 2-O-[3-Acetyl-1-(Trimethylsilyl)-1h-Indolyl]-D-Glucose ($C_{32}H_{62}N_2O_7$; 1.38%).

Table 3. Relative abundance (%) of the main groups of pyrolysis products of *Adenostemma*.

Metabolite Group	N	Alk	MA	C	Al	Alc	Ar	Ph	Lp	TS
Al1	13,39	6,94	6,11	5,44	0	2,61	3,68	44,84	11,9	5,08
Al2	27,97	2,76	0	0	0	3,93	10,71	0	44,22	10,41
Al3	11,88	11,24	3,93	1,51	1,41	0	12,59	4,37	29,62	23,46
Ap1	2,55	0	1,08	4,69	2,01	0	2,37	72,45	14,88	0
Ap2	3,1	0	0	0	5,25	8,66	0	62,74	18,51	1,73
Ap3	12,06	4,27	1,63	1,41	7,21	17,22	13,72	7,42	18,23	16,82

Note: Code of studied species as in Table 1. N, N-bearing compounds (except alkaloids); Alk, Alkaloids; MA, multi-origin aliphatic compounds with C≤6; C, furan originated from carbohydrates. pyran and cyclopentene derivatives; Al. aliphatic compounds with C>6; Alc, Alcohol; Ar, aromatic compounds (except phenolic compounds); Ph, phenolic compounds; Lp, compounds originated from lipids (except terpenoid, steroid); TS, terpenoid-steroid.

Those compounds support the healing of wound or infection, antioxidant and also act as antibacterial agents. Naturally occurring phenolic compounds have been shown to scavenge active oxygen species and to effectively prevent oxidative cell damage [32]. Inhibitory effects of phenolic compounds containing allyl groups were similar to those of flavonoids [33]. 2,6-diphenyl-piperidine, and 5,10-diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2- a;1 -d] pyrazine (a member of alkaloids) also acts as antifungal agent [34]. A considerable amount of 3-methylpyridine is used as a starting material for pharmaceuticals and agrochemicals [42] and 4-cyano-3-methylisoquinoline as potential antimalarial agents [35].

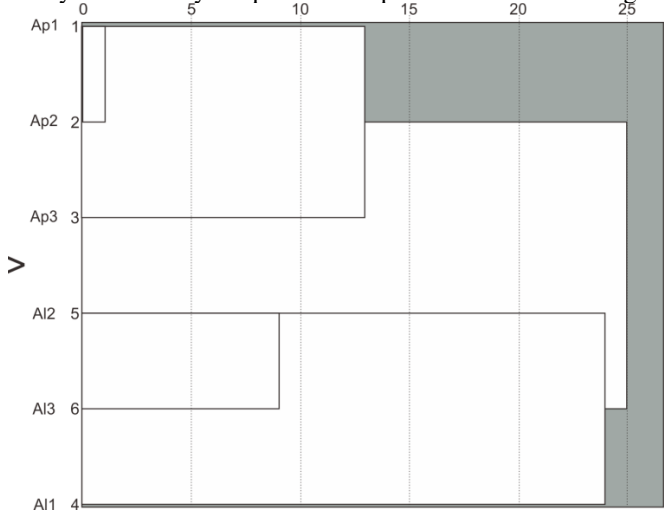


Fig. 2. Cluster analysis of amounts of pyrolysis products of *Adenoetemma*.

Note : Ap1: root of *A platyphyllum*; Ap2: stem of *A platyphyllum*; Ap3: leaves of *A platyphyllum*; Al1: root of *A lavenia*; Ap2: stem of *Alavenia*; Ap3: leaves of *A lavenia*

3.3 *Cluster Analysis*

Cluster analysis of pyrolysis products indicates not similitary of major chemical compounds of the studied *Adenostemma* species (Fig. 2). For example, despited the evident similarity in plants organs composition, the *A. platyphyllum* species were grouped together. For example, despite the evident similarity in plants organs composition, the *A. platyphyllum* species were grouped together. The decisiveness of this classification is reflected in the dendrogram (fig. 2). The initial splitting of the tree forms to two clusters. The top contains *A. platyphyllum*. The bottom contains *A. lavenia*. The *A. platyphyllum* roots and *A. platyphyllum* stems are each more similar than the *A. platyphyllum*leaves. The chemical compounds in chemical taxonomy of *Adenostemma* can be seen in Appendix 1.

4. Conclusions

The chemical compounds *A. lavenia* and *A. platyphyllum* are significantly different. Pyrolysis GCMS showed that the major compound of *A. platyphyllum* is aromatic compounds (phenolic) with a concentration of 26.4% and *A. lavenia* is originated lipids and terpenoid steroid with a concentration of

29.7% and 15.8%. Chemical compounds of *A. platyphyllum* and *A. lavenia* as potential antimicrobial, antioxidant and anti-inflammation agent.

References

[1] Orchard AE. 2011. A review of Australian Adenostemma J.R.Forst. & G.Forst. (Asteraceae: Eupatorieae). *Telopea* 13(1–2): 341–348.
[2] Singh G. Passari AK. Singh BP and Kumar NS. 2017. Traditionally Used Medicinal Plants Belongs to Family Asteraceae for the Treatment of Cancer in Mizoram. Northeast India
[3] Valentin-Silva A. Godinho MAS & Vieira MF. 2016. Life history of *Adenostemma brasilianum* (Eupatorieae. Asteraceae): A Psychophilous Herbaceous Species of the Brazilian Atlantic Forest Understory. Journal of the Torrey Botanical Society 143(1): 87–92. 2016.
[4] Amjad MS. Arshad M. Page S. Qureshi R. dan Mirza SN. 2017. Floristic composition. Biological spectrum and phenological pattern of vegetation in the subtropical forest of Kotli District. AJK. Pakistan. *Pure Appl. Biol*. 6 (2): 426-447. doi. 10.19045/bspab.2017.60043.
[5] Sivaraj N. Pandravada SR. Venkateswaran K. dan Dikshit N. 2017. Ethnic Medicinal Plant Wealth of Eastern Ghats: Status. Knowledge Systems and Conservation Strategies. *Int. J. Curr. Res. Biosci. Plant Biol.* 4 (1). 83-101. DOI 10.20546/ijcrbp.2017.401.010.
[6] Castro. AMO. 2017. Knowledge and Traditional Use of the Phytomedicinal Resource of the Yurumanguí River Community. Buenaventura District. *Thesis.* Facultad de Ciencias Contables Económicas y Administrativas Maestría en Desarrollo Sostenible y Medio Ambiente Manizales. Colombia. (In Spanish)
[7] Godinho MAS. Alvarenga EM. & Vieira MF. 2011. Germination and Seed Quality of Adenostemma brasilianum. Revista Árvore. Viçosa-MG. 35 (6): 1197-1205. (In Portoguese)
[8] Cheng PC. Hufford CD & Doorendos NJ. 1979. Isolation of 11-Hydroxylated Kauranic Acids from *Adenostemma lavenia*. *Journal of Natural Products.* 42 (2): 183 – 186.
[9] Shimizu S. Miyase T. Umehara K & Ueno A. 1990. Kaurane-Type Diperpenes from *Adenostemma lavenia* O. KUNTZE. Chem. Pham. Bull. 38 5 : 1308 – 1312 1990.
[10] Bardon A. Montanaro S. Catalan CAN. Diaz JG. dan Werner Herz W. 1996. Kauranes and Related Diterpenes from *Adenostemma brasilianum*. *Phytochemistry*. 42. (2): 479 484. 1996.
[11] Fauzan A and Walid M. 2014. Study of Legetan Warak Utilization as Natural Food Preservative. Research Report. BAPPEDA Pekalongan City. Pekalongan (In Bahasa Indonesia).
[12] Yong-li Y. Shou-jun G. Rui-jun MA & Zui-luan W. 2007. Chemical Composition of the Volatile Oil in *Adenostemma lavenia* L.O.Kutze. *J. Tropical and Subtropical Botany*. 15 (4): 355 – 358.
[13] Fatimah S. Handarto BM. 2008. The Influence of Planting Media Composition Toward Growth and Plant Result of *Andrographis paniculata* Ness. *Embryo* 5 (2): 133-148. (In Bahasa Indonesia)
[14] Gil-Munoz R. Fernández-Fernández JI. Crespo-Villegas O dan Garde-Cerdán T. 2017. Elicitors used as a tool to increase stilbenes in grapes and wines. *Food Research International*. 98 (2017): 34-39. DOI 10.1016/j.foodres.2016.11.035.
[15] Raskin I. Ripoll C. 2004. Can an apple a day keep the doctor away? Current Pharmaceutical Design. 10:1-9
[16] Stanchiu 2017. Climatic conditions influence emerging mycotoxin presence in wheat grown in Romania – A 2-year survey. *Crop Protection*. 100 (2017): 124-133.
[17] Achakzai AKK. Achakzai P. Masood A. Kayani1 SA dan Tareen RB. 2009. Response Of Plant Parts And Age On The Distribution Of Secondary Metabolites On Plants Found In Quetta. *Pak. J. Bot.*41 (5): 2129-2135.
[18] Figueiredo AC. Barroso JG. Pedro1 LG and Scheffer JJC. 2008. Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour And Fragrance J. 23 (2008): 213–226. DOI: 10.1002/ffj.1875.
[19] Figueiredo AC. Barroso JG. Pedro1 LG and Scheffer JJC. 2008. Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour And Fragrance J. 23 (2008): 213–226. DOI: 10.1002/ffj.1875.
[20] Wink M. 2010. Biochemistry of Plant Secondary Metabolism. Wiley-Blackwell. West Sussex, United Kingdom
[21] Klavina L. Bikovens O. Steinberga I. Maksimova V and Eglite L. 2012. Characterization of chemical composition of some byrophytes common in Latvia. J Enviromental and Experimental Biology (2012 10: 27 – 34.
[22] AOAC. 1990. Official Methods of Analysis. 15th Edn. Association of Official Analytical Chemists Washington, DC, USA.

[23] Husain J., Khan A.L., Rehman N, Hamayun M., Shah T., Nisar M, Bano T, Shinwari Z.K and Lee I.J. 2009. Proximate and nutrient analysis of selected vegetable species: A case study of Karak region, Pakistan. African Journal of Biotechnology. 8 (12): 2725-29.
[24] Malik J., Santoso A., Mulyana Y., and Ozarska O. 2008. Characterization of Merbau Extractives as a Potential Wood-Impregnating Material. BioResources 11(3): 7737-53.
[25] Steinbach M., Ertoz L., Kumar V. 2003. Challenges of clustering in high dimensional data. University of Minnesota Supercomp. Inst. Res. Report 2013: 1-33
[26] Downs G.M., Barnard J.M. 2003. Clustering methods and their uses in computational chemistry. Rev. Comput.Chem. 18: 1–40.
[27] Anderberg, MR. 2014. Cluster Analysis for Applications: Probability and Mathematical Statistics: A Series of Monographs and Textbooks. Academic Press, New York.
[28] Hervada-Sala C. and Jarauta-Bragulat E. 2004. A program to perform Ward`s clustering method on several regionalized variables. Comp. Geosci. 20: 881–886.
[29] Mandal V, Mohan Y & Hemalatha S. 2007. Microwave assisted extractionan innovative and promissing extraction tool for medicinal plant research. Pharmacognosy Reviews 1:7-18
[30] Zheng J.L. 2007. Bio-oil from fast pyrolysis of rice husk: Yields and related properties and improvement of the pyrolysis system. J. of Analytical and Applied Pyrolysis 80(1): 30-35.
[31] Mullen, C.A., and Boateng, A A. 2008. Chemical composition of bio-oils produced by fast pyrolysis of two energy crops. Energy and Fuels 22(3): 2104-2109.
[32] Okuda, T.; Y. Kimura, T. Yoshida, T. Hatano, H. Okuda, and S. Arichi. 1983. Studies on Activities of Tannins and Related Compounds from Medical Plants and Drugs. I. Inhibitory Effects on Lipid Oxidation in Mitochondria and Microsomes of Liver. Chem. Pharm. Bull. 31:1625–31.
[33] Ogata, M.; Hosho, M.; Shimotohno, K.; Urano S.; and Endo, T. 1997. Antioxidant Activity of Magnolol, Honokiol, and Related Phenolic Compounds. JAOCS. 74(5):557-562.
[34] Li, H.; Liu, L.; Zhang, S.; Cui, W.; and Jiaping, LV. 2012. Identification of Antifungal Compounds Produced by Lactobacillus casei AST18. Curr Microbiol (2012) 65:156–161.
[35] Buskes, M.J.; Harvey, K.L.; Richards, B.J.; Kalhor, R.; Christoff, R.M.; Gardhi, C.K.; Littler, D.R.; Cope, E.D.; Prinz, B.; Weiss, G.E.; O'Brien, N.J.; Crabb, B.S.; Deady, L.W., Gilsonb, P.R.; and Abbott, B.M. 2016. Antimalarial activity of novel 4-cyano-3-methylisoquinoline inhibitors against Plasmodium falciparum: design, synthesis and biological evaluation. Organic &Biomolecular Chemistry. 14(20):4617-39.

Appendix 1. Peak assignments and relative abundance (%) of pyrolysis products of *Adenostemma*.

R-Time	Name	MG	WM	Formula	Al1	Al2	Al3	Ap1	Ap2	Ap3
18,643	2-Methyl-8-Propyldodecane	Al		C16H34						1,69
21,225	4,5-Nonadiene (Cas)	Al		C9H16					5,25	
21,333	Cycloundecene	Al	152	C11H20			1,41			
22,758	1-Chloropentadec-11-Yne	Al		C15H27Cl						1,82
22,925	Cyclohexene, 3-Methylene-4-(1,2-Propadienyl)	Al		C10H12						1,54
14,042	N-Heptanal	Al		C7H14O				2,01		
18,265	N-Nonanal	Al		C9H18O						2,16
3,22	(O-D)Ethenol	Alc		C2H3DO					2,77	4,69
14,782	3,5-Heptadien-2-Ol, 2,6-Dimethyl-	Alc	140	C9H16O		1,83				
16,396	Hexa-2,4-Diyne-1,6-Diol	Alc		C6H6O2						1,52
19,438	3-Nonyn-2-Ol (Cas)	Alc		C9H16O						1,93
20,086	Cis-9-Octadecen-1-Ol	Alc		C18H36O						3,86
20,358	Octilin	Alc		C8H18O					5,89	
20,395	Phytol	Alc		C20H40O						3,77
21,229	(Z)6-Pentadecen-1-Ol	Alc	226	C15H30O	2,61					
24,076	N-Eicosanol	Alc	298	C20H42O		2,1				
18,875	Dodecan-7-Ol	Alc		C12H20O						1,45
16,196	5h-1-Pyrindine	Alk	117	C8H7N			5,07			
17,017	3-Methylindole	Alk	131	C9H9N		0,93				1,62
17,064	D-Glucose, 4-O-[3-Acetyl-1-(Trimethylsilyl)-1h-Indolyl]-	Alk	726	C32H62N2O7Si5			2,14			
17,859	D-Glucose, 2-O-[3-Acetyl-1-(Trimethylsilyl)-1h-Indolyl]-	Alk		C32H62N2O7						1,38
18,364	6,7-Dihydro-3-Nitro-5h-Cyclopenta[B]Pyridin-2(1h)-One	Alk	180	C8H8N2O	2,94					
21,523	5,10-Diethoxy-2,3,7,8-Tetrahydro-1h,6h-Dipyrrolo[1,2-A;1',2'-D];	Alk	250	C14H22N2O2	4,00					
22,245	1-Cyano-3methylisoquinoline	Alk	168	C11H8N2		1,83				
23,466	6-Benzyl-3-Methyl-6,7-Dihydro-4h-Isoxazolo[5,4-C]	Alk		C14H14N2O2						1,27
14,55	3,5-Xylenol	Ar		C8H10O						1,68
15,444	Hyacinthin	Ar		C8H8O						1,83
15,465	O-Tolualdehyde	Ar		C8H8O				0,66		
16,413	4-Formylcyclohexene	Ar		C7H10O				1,71		
19,923	Mixture Of 1-Methylidene-4a.Alpha.-Methyl-1,2,3,4,4a,9,10,10a.Alpha.Octahydro	Ar		C16H20						2,08
21,215	Biphenyl, 3,4-Diethyl	Ar	166	C12H22		7,54	3,41			8,13
21,699	5-Beta,8-Beta-Epoxy-3,5,8,8a-Tetrahydro-1h-2-Benzopyr-	Ar	150	C9H10O2	3,68					
22,297	Spiro[Cyclobutane-1,1'(2'h)-Phenanthrene],	Ar	240	C18H24			2,54			
22,396	3,6-Dimethylphenanthrene	Ar	206	C16H14		3,17				
22,451	Anthracene, 9,10-Dimethyl- (Cas)	Ar	206	C16H14			4,95			
23,184	1-Iodo-4-Phenylbicyclo(2	Ar	312	C14H17I			1,69			
9,38	Cyclopentenone	C		C5H6O				0,86		
12,934	Corylon	C	112	C6H8O2	5,44			2,43		1,41
12,936	2,5-Methano-2h-Furo[3,2-B] -8-One, Hexahydro-	C	154	C8H10O3			1,51			

13,326	3-Ethyl-2-Hydroxy-2-Cyclopenten-1-One	C		C7H10O2				1,4		
17,083	2-Methyldecane	Lp	156	C11H24		0,67				
17,884	Carbazic Acid, 3-Pentylidene-, Ethyl Ester	Lp	168	C12H24			3,04			
18,235	Oleic Acid	Lp	282	C18H34O2		1,92				1,55
18,609	N-Dodecane	Lp	170	C12H26		0,81				
19,242	Adacene 12	Lp	168	C12H24		0,53	3,24			
20,048	Z9-Dodecenylacetate	Lp	226	C14H26O2		0,7				
20,123	Trans-2-Dodecenal	Lp	180	C12H20O			2,28			
21,094	Palmitic Acid	Lp	256	C16H32O2	3,92	3,21	4,38	9,83	8,58	5,05
21,234	Cycloundecene, (Z)-	Lp		C11H20						3,15
21,265	1,12-Tridecadiene	Lp	180	C13H24			5,11			
21,316	Ambrettolide	Lp		C16H28O2						1,84
21,477	1a,9b-Dihydro-1h-Cyclopropa[A]Anthracene	Lp	192	C15H12		4,3	5,75		5,97	
21,514	1-Methylanthracene	Lp		C15H12						5,16
21,908	9,12-Octadecadienoyl Chloride, (Z,Z)- $$	Lp	298	C18H31ClO		1,43				
22,078	1-Chlorooctane	Lp	148	C8H17Cl		1,85				
22,525	Epoxycyclododecane	Lp	182	C12H22O		17				
22,583	Stearic Acid	Lp	284	C18H36O2	3,24	1,36		5,05		
22,958	Cis-3-Undecene-1,5-Diyne	Lp	146	C11H14		2,29				
22,982	Cyclododeca-1,5-Dien-9-In	Lp	160	C12H16			3,11			
23,058	2-Octenoic Acid (Cas)	Lp	142	C8H14O2		1,29				
23,228	1-Chlorooctadecane	Lp	220	C17H16		2,8	2,71			
23,333	1-Chlorooctadecane	Lp	288	C18H37Cl		1,32				
23,883	Cis-Octadec-9-Enal	Lp	266	C18H34O	2,73				1,99	1,48
23,899	Trans-Dodec-5-Enal	Lp	182	C12H22O		2,79				
24,062	Cyclohexyleicosane	Lp	364	C26H52	2,01				1,97	
2,804	Cyclopropyl-Cis-1,2,3-D3-Methanol	MA	72	C4H5D3O			3,93			
3,298	2,3-Epoxybutane	MA		C4H8O				0,51		
11,368	6-Oxa-Bicyclo[3,1,0]Hexan-3-One	MA	98	C5H6O2	2,23			0,57		
13,879	N-Pentanal	MA	86	C5H10O	3,88					1,63
19,645	2-Imidazolidinone, 1,3-Diethenyl-	N	138	C7H10N2O			4,03			
3,308	Methanamide	N	45	CH3NO	6,62					
3,317	Ammonium Carbamate	N	61	CH3NO2		7,59				
5,48	Sym-Dimethylhydrazine	N		C2H8N2				2,55		
16,152	7-Cyano(15n)-Cycloheptatriene	N	117	C8H7 15N		1,97			2,07	
16,176	1-Methyl-2-Cyanobenzene	N		C8H7N						3,79
17,175	2-Amino-4,5-Dimethyloxazole	N		C5H8N2O						2,09
19,806	N-Phenyl-N'-Furaldehyde Hydrazone	N	186	C11H10N2O	2,19					
19,819	1-Cyclopentene-1-Carboxamide	N		C12H13NO						1,26
20,158	1,8-Octanediamine	N	144	C8 H20 N2		3,04				
20,608	Pentadecanonitrile	N	223	C15H29N		1,18	1,71			
20,642	Palmitonitrile	N		C16H31N						1,16
21,355	1,4-Diaza-2,5-Dioxo-3-Isobutyl Bicyclo[4,3,0]Nonane	N	210	C11H18N2O2	2,49	5,47	5,22			
22,78	Amide 16	N	255	C16H33NO	2,09		3,41		1,03	
24,46	Oleoamide	N	281	C18H35NO		8,72	1,54			3,76
12,55	Phenol (Cas) Izal	Phenol	94	C6H6O			1,85			2,62
13,458	O-Cresol	Phenol	108	C7H8O	2,83		2,52	1,51		1,12
13,658	Guaiacol	Phenol	124	C7H8O2	2,91				1,97	
13,716	Phenol, 4-Methoxy- (Cas) Hqmme	Phenol		C7H8O2				1,94		
13,746	P-Cresol	Phenol	108	C7H8O	4,47					1,9
14,773	O-Ethylphenol	Phenol	122	C8H10O	2,19					
14,787	M-Ethylphenol	Phenol		C8H10O					2,48	1,78
14,832	2,3-Dimethylphenol	Phenol		C8H10O				0,83		
14,897	2-Methoxy-4-Methylphenol	Phenol		C8H10O2				1,04		
15,778	2,5-Dimethoxytoluene	Phenol		C9H12O2				0,45		
16,161	Carvacrol	Phenol	150	C10H14O	3,52			1,59		
16,483	2,6-Dimethoxyphenol	Phenol	154	C8H10O3	3,29			1,99	1,99	
16,625	5-Propyl-Guaiacol	Phenol		C10H14O2				0,32		
17,326	1,2,3-Trimethoxybenzene	Phenol	168	C9H12O3	2,33					
17,391	2,5-Dimethoxybenzyl Alcohol	Phenol		C9H12O3				5,98		
17,458	Aceteugenol	Phenol		C12H14O3				3,89		
18,028	Toluene, 3,4,5-Trimethoxy-	Phenol		C10H14O3				0,63		
18,22	1,6-Anhydro-Beta-D-Glucopyranose; Levoglucosan	Phenol	162	C6H10O5	8,61				6,27	
18,229	1-(4-Hydroxy-3methoxy	Phenol		C10H12O3				6,73		
18,369	Methyl Meta-Methoxybenzyl Acetate	Phenol		C10H12O3					1,04	
18,657	4-Allyl-2,6-Dimethoxyphenol	Phenol	194	C11H14O3	2,19			14,2	2,01	
19,709	Myristic Acid	Phenol		C14H28O2					6,68	
19,763	Acetosyringone	Phenol	196	C10H12O4	1,84				2,49	
19,866	3,4,5-Trimethoxybenzaldehyde	Phenol		C10H12O4				5,76		
19,905	Coniferyl Alcohol	Phenol	180	C10H12O3	2,88			13,7	6,75	
19,983	2,4-Hexadienedioic Acid, 3,4-Diethyl-, Dimethyl Ester	Phenol	226	C12H18O4	1,93				3,71	
20,583	2-Methyl-4,6-Bis(Ethylamin	Phenol		C8H15N5					2,66	
21,707	1h-2,8a-Methanocyclopenta[A]Cyclopropa[E]Cyclodecen-11-One, 1a,2,5,5a,6,	Phenol		C20H28O6					6,46	
22,4	Linoleic Acid	Phenol	280	C18H32O2	3,32			11,9		
22,417	Tetradecahydroanthracene	Phenol		C26H48					11,8	
22,483	Methyl Linolenate	Phenol		C19H32O2					4,11	
22,525	Mannit, 5-Phenylpent-1-Yl-	Phenol		C17H28O6					2,3	
22,556	N-Hexatriacontane	Phenol	507	C36H74	2,53					
12,668	L-Limonene	TS	136	C10H16		2,31	4,68			2,62
20,195	3A,9B-Dimethyl-1,2,3A,4,5,9B-Hexahydro-Cyclopent	TS		C15H18O						1,65
20,551	Isopropyl Biphenyl	TS	196	C15H16		2,87	1,86			
21,803	N-Pentacosane	TS	352	C25H52		1,8	4,45			
22,551	Cis,Cis,Cis-8,11,14eicosatrienoic Acid	TS	306	C20H34O2			10,6			12,6
24,211	Spiro[Androst-5-Ene-17,1'-Cyclobutan]-2'-One,3-hydroxy	TS	328	C22H32O2		1,35	1,88			
27,529	17-Acetoxy-19 kauranal	TS		C22H34O3					1,73	
38,938	Stigmasta-5,22dien	TS	454	C31H50O2	5,08	2,08				

Note: MG, Metabolite group; WM, weight of molecular; Al_1, root of *Adenostemma lavenia*; Al_1, root of *Adenostemma lavenia*; Al_1, root of *Adenostemma lavenia*; N, N-bearing compounds (except alkaloids); Alk, Alkaloids; MA, multi-origin aliphatic compounds with C≤6; C, furan originated from carbohydrates. pyran and cyclopentene derivatives; Al. aliphatic compounds with C>6; Alc, Alcohol; Ar, aromatic compounds (except phenolic compounds); Ph, phenolic compounds; Lp, compounds originated from lipids (except terpenoid, steroid); TS, terpenoid-steroid.