Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(4):866-872

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Toxicity and phytochemistry test of methanol extract of several plants from papua using *Brine Shrimp Lethality Test* (BSLT)

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ABSTRACT

Papuan endemic plants such as Evodia suaveolens Scheff (Ruteceae), Piper methysticum G. Forst. (Piperaceae), Xanthosthemon novaguineense Valet. (Myrtaceae) and Macaranga aleuritoides F. Muell. (Euphorbiaceae) are used as medicinal plants. Some research indicates that medicinal plants can be used as a botanical insecticide. The objective of this research was to determine the toxicity of methanol extract and plant phytochemistry content. Toxicity test extracts was assessed using shrimp lethality as an indicator of toxicity. The results showed that the toxicity test using the method BSLT methanol extract of P. methysticum plant obtained LC_{50} = 99.05 ppm and E. suaveolens LC_{50} = 131.34 ppm is more toxic than X. novaguineense extract LC_{50} = 5,372,86 ppm and M. aleuritoides LC_{50} = 6,710,94 ppm. Phytochemistry components contained in the plant P. methysticum and E. suaveolens are alkaloids, flavonoids, tannins and saponins, whereas plants X. novaguineense and M. aleuritoides contains flavonoids, tannins and saponins. Among the four plant extracts, the most toxic plants are P. methysticum and E. suaveolens and can be used as a botanical insecticide.

Keywords: Toxicity, Phytochemistry, *E.suaveolens, P. methysticum, X. novaguineense, M. aleuritoides*

INTRODUCTION

Several Papua endemic plants such as *Evodia suaveolens* Scheff, *Piper methysticum* G. Forst, *Xanthosthemon novaguineense* Valet. and *Macaranga aleuritoides* F.Muell. often used as a medicinal plant for the Papuan people. The chemicals contents of the medicinal plant believed to be used as a botanical insecticide.

E. suaveolens known as plant repel mosquitoes because its contain active ingredients of *Evodiamine* and *rutaecarpine* that included in alkaloids [1], the oil was distilled from the leaves of plants *E. suaveolens* contain linalool (46%) and *a-pinene* (13,26%) [2], in which *linalool* is known as a repellent of mosquitoes. *P. methysticum* by Marind tribal population in Merauke region of Papua as a plant in alcoholic or have quality as narcotic because it is thought to contain chemical compounds such as *kawain, dihidrokawain* (marindinin), *metistisin, dihidrometistisin* and *yangonin* that as the sedative [3-4].

P. methysticum very effective as an antibiotic, antiseptic, antimicrobial and as narcotics and effective for controlling bacteria, such as *Alternaria solani, Botrytis cinerea, Ceratocystis ulmi, Sclerotinia fructicola* [1]. *X. novaguineense* Valet. used by the community for the purpose of home building materials, caused *X. novaguineense* classified as wood resistant to attack by wood destroying that subterranean termites, wood borers in the sea, the white-rot fungi and brown-rot fungi [5]. Extract of *X. novaguineense* can inhibit the growth of termites and wood-rot fungi [6]. *M. aleuritoides* used in traditional medicine such as diarrhea, injury and cough. Research results showed that

Macaranga spp produced flavonoid phenolic compounds and stilbene which has bioactivity as antitumor, anticancer, antiviral, antimicrobial and antioxidant [7]. *M. triloba* as potential anti-HIV drugs and species *M. peltata* can control insects *Dysdercus cingulatus* [1,8].

Brine Shrimp Lethallity Test (BSLT) was used as bioassay method in screening for active compounds or active extracts from natural materials [9-10]. BSLT method is used to detect the presence of toxic compounds, and are used determine the LC_{50} value of the active compound [11].

The potential for active compounds possessed some of the plants from Papua, as well as their toxicity is not known, it is necessary to investigate the toxicity and phytochemistry content of the methanol extract of leaves of plant *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. aleuritoides* using *Brine Shrimp Lethality Test* (BSLT).

EXPERIMENTAL SECTION

Sample preparation

Plant material such as *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. aleuritoides*, were collected from public forests in Jayapura regency, City of Jayapura and Merauke regency in Papua province, then dried and mashed to the ground to fine powder 30 mesh.

Extraction of plant material

Fine powder 250 g of plant material was macerated with 95% methanol at room temperature for 3 x 24 hours, repeated until maceration obtained translucent color. Extract of obtained was concentrated to obtain further combined and filtered using filter paper, and then the solvent removed using a rotary vacuum evaporator at 40 °C to obtain a concentrated methanol extract of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. Aleuritoides*.

Qualitative phytochemical test

Phytochemistry test conducted to determine the content of phytochemistry from plant leaves of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. aleuritoides* by identifying alkaloids, flavonoids, tannins and saponins compounds.

Test of alkaloid content

Alkaloid test performed by the method of Meyer and Dragendroff. Sample solution in methanol (0.3 g) is inserted in a porcelain cup and then add 5 mL of 2 M HCl, heated over a waterbath for 3 min, stirring and cooled. Then added 0.5 g of NaCl and stired and filtered. The filtrate obtained was added 2 M HCl as much as 5 ml and then separated into 3 sections namely A, B and C. The filtrate A plus Mayer reagent, filtrate B coupled with Dragrendroff reagent and the filtrate C to form. When precipitation indicates that the sample contains alkaloids, with reagent Meyer gives a white precipitate, and Dragendorff reagent give an violet precipitate.

Test of Flavonoids Content

To determine the presence of flavonoid compounds is carried test of Bate-Smith and Metcalf test and Test of Wilstater. Sample solution in methanol (0.3 mg) was shaken with 3 ml of n-hexane repeatedly until the n-hexane extract colorless or clear. The residue obtained was dissolved in 10 ml of ethanol was then filtered, the filtrate obtained was divided into three, namely A (blank), B (test of Bate-Smith and Metcalf) and C (Wilstater test). The filtrate B was added 0.5 mL of concentrated HCl and observe the color change and then heated and observed color change occurs. When the color slowly changed into a bright red/purple, then showed the presence of compounds leucoantosianine/flavonoids (compared to the blank). The filtrate C was added 0.5 mL of concentrated HCl and 5 pieces of magnesium plate observe the color change, then add distilled water and add 1 ml of n-butanol produce yellow, orange, red or blue colour, the compound formed was included the flavonoid [12].

Test of Tannins Content

To determine the presence of tannin compounds is carried of Ferrikloride Test. Sample solution in methanol (0.3 g) was added 10 ml of distilled water, heated, stirred and cooled then added 4 drops of 10% NaCl stirred and filtered. The filtrate was divided into two parts, namely A (blank), and B (test of Ferrichloride). The filtrate B added a few drops of 5% $FeCl_3$ observed color change if there is a change blackish green color indicates the presence of tannins

Test of Saponins Content

Test of saponin was conducted using the Forth method by entering 2 ml sample into a test tube and added 10 ml of distilled water and shake for 30 seconds and observe what happens. If the foam is formed solid (not lost for 30 seconds) the identification showed the presence of saponins.

Toxicity tests using the method of Brine Shrimp Lethallity Test (BSLT)

Method of Meyer [9], is used to study the toxicity of the general sample using shrimp eggs (*A. salina Leach*). *Brine Shrimp Lethality Test* (BSLT) is one of the methods bioactive compounds present in natural materials using shrimp larvae (*A. salina*). Known toxicity properties based on the number of larvae mortality [13]. An extract is said to be toxic to *A. salina* if it has a value of LC_{50} (lethal concentration to 50% larval shrimp) less than 1000 µg/ml.

The hatching of Shrimp larvae

Prepared shrimp vessel for hatching eggs which have been filled with sea water 1,500 ml, with pH of 7.7 and 87.6% salinity levels, place the lamp to warm temperatures in vessel of hatching and fed air by using the aerator. Inserted into the sea water of 0.3 g shrimp eggs for hatching. Vessel hatching eggs covered with aluminum foil, and the lights turned on for 48 hours to incubate the eggs. After 48 hours of shrimp eggs will hatch into larvae and ready for use. Shrimp larvae that will be used for testing were taken using a pipette.

Preparation of sample solution that will be tested.

Methanol extract of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. aleuritoides* that will be tested each made in concentrations of 0, 10, 100, 200, 500 and 1000 ppm in sea water. When the methanol extract insoluble added 2 drops of DMSO (dimethyl sulfoxide).

Procedure of Toxicity Test Methods using BSLT

Pipette 100 µL seawater containing as many as 20 larvae shrimp, then put into a test tube. Added solution of methanol extract of the sample concentrations of 10, 100, 200, 500 and 1000 µg/mL and performed 3 repetitions. To control performed without the addition of methanol extract. Test tube and placed under light irradiation was left for 24 hours, then counted the number of larvae that die and are still alive and then used to determine the level of toxicity (LC_{50}) and toxicity categories according to Table 1.

Table 1. Relationship between LC_{50} and Toxicity Category

Categories	LC_{50} values
Supertoxic	≤ 5 mg/kg
Very toxic	5-50 mg/kg
Toxic	50-500 mg/kg
Toxic medium	0.5-6 g/kg
Mild toxic	5-15 g/kg
Practically non-toxic	> 15 g/kg

Observations were made after 24 hours to calculate the percentage of mortality shrimp larvae *A. salina*. Mortality data are used to calculate the value of Lethal Concentration 50 (LC_{50}). The graph is made with a log concentration as the x-axis on mortality as the y-axis. The LC_{50} value is the concentration of a substance which causes the death of 50% obtained by using linear regression equation $y = a + bx$. A substance said to be active or toxic when LC_{50} values < 1000 µg/ml to extract and < 30 µg/ml for a compound.

RESULTS AND DISCUSSION

Phytochemistry screening

Components that contained in the methanol extract of the leaves of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. aleuritoides* analyzed group of secondary metabolic compounds to color test with several classes of reagents for alkaloids, flavonoids, tannins and saponins. The result of phytochemistry screening of methanol extracts are presented in Table 2.

Table 2. Phytochemistry crude methanol extract of plants leaves of *P. methysticum* G. Forst., *E. suaveolens* Scheff, *X. novaguineense* Valet. and *M. aleuritoides* F.Muell

Test of Chemistry Content	Plants			
	P. methysticum	***E. suaveolens***	***X. novaguineense***	***M. aleuritoides***
Alkaloids				
- Mayer reagent	++	++	-	-
- Dragendroff reagent	++	++	-	-
Flavonoids				
- Bate-Smith and Metcalf test	++	++	++	++
- Wilstater test	++	++	++	++
Tannins	++	++	++	++
Saponins	++	++	++	++

notes: *++ = there is a strong positive* *- = negative*

Based on the results of phytochemistry screening revealed that crude methanol extract of *P. methysticum* plant leaves and *E. suaveolens*, contains alkaloids. This is evident from the precipitate. Mayer reagent will react with alkaloids and form a white precipitate and Dragendorff reagent forms a precipitate orange [14].

Positive results of alkaloid with Mayer reagent characterized by the formation of a white precipitate. Crude methanol extract of *P. methysticum* plant and *E. suaveolens* positive for alkaloids as a white precipitate formed while the crude methanol extract of the leaves of plants *X. novaguineense* and *M. aleuritoides* no formed white precipitate.

Positive results alkaloid with Dragendorff reagent characterized by the formation of brown to yellow precipitate. Methanol extract of *P. methysticum* plant leaves, *E. suaveolen* positive contain alkaloids as forming yellowish brown precipitate, while the methanol extract of the leaves of plants *X. novaguineense* and *M. aleuritoides* not contain alkaloids such as because both of its does not form a precipitate brown to yellow. Alkaloids contain nitrogen as part of a cyclic system and contain varying substituent groups are like amine, amide, phenol, and methoxy so alkaloids are semipolar [15]

Testing flavonoid compounds by using test of Bate-Smith and Metcalf marked discoloration ethanol extract bright red to brownish red. Crude extracts of *P. methysticum* plant, *E. suaveolens*, *X. novaguineense*, and *M. aleuritoides* positive contain flavonoids because methanol extracts of these plants that change color to red.

Testing flavonoid compounds using Wilstater test conducted by reacting and ethanol extracts with magnesium using concentrated HCl. Test ethanol serves to dissolve the flavonoid compounds that contained in the extract of leaves of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. Aleuritoides*. Reduction with magnesium and concentrated hydrochloric acid produces a red color in flavonols, flavanones, flavanonol and Xanthone. Based on tests that have been done, the extract of leaves of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. aleuritoides* showed positive results with test Shinoda (Mg+HCl) because it produces a red colored solution. This shows that the plant extract of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. aleuritoides* contain secondary metabolites compound in flavonoid groups. Complex that red colored resulting from coordination covalent bond between magnesium ions with phenolic OH group of flavonoid compounds. According to Markham, Flavonoids are polar because of having bonding with the sugar group [12].

Based on the screening results of phytochemistry tannin compound, it is known that the plant leaf extracts of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. aleuritoides* containing tannin compounds. Color changes that occur during the addition of 1% $FeCl_3$ solution became blackish green color. In addition solution $FeCl_3$ of 1% is expected to react with one hydroxyl group on tannin compound. $FeCl_3$ reagent used extensively to identify phenolic compounds including tannins [14]. The results of tests performed on the test tube using $FeCl_3$ solution indicates the onset of a green color. The test results showed that the plant extract of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. aleuritoides* positive for class of tannin compounds because plant extracts that given $FeCl_3$ solution shows a color change to green. Tannins group are phenolic compounds that tend to dissolve in water and polar solvents [15].

From the screening results of phytochemistry leaf extract of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. aleuritoides* positive contain saponin compounds. This is evident from the resulting stable foam. According to Robinson [14], a compound having polar and nonpolar groups are active surface so that when shaken with water, saponins can form micelles. In the micelle structure, polar groups facing outward while non polar groups facing inwards. This condition looks like foam. Saponins are triterpene glycosides which have tended polar because its glycosides bonding [15].

Toxicity tests using *Brine shrimp Lethality Test* (BSLT)

Toxicity tests of crude leaf extracts of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. aleuritoides* conducted to determine the level of toxicity of the extracts against larvae shrimp *A. Salina*. The test results showed that the leaf extract of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. aleuritoides* showed that at different concentration levels will have an impact on mortality and larval toxicity of this case is shown in Table 3 and Figure 1.

Methanol extract of plant leaves of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. Aleuritoides* significant effect on mortality at different concentrations. Methanol extract of leaves of *P. methysticum* and *E. suaveolens* has the highest mortality rate up to 100% compared with the methanol extract of the leaves of *X. novaguineense* and *M. aleuritoides*.

Mortality of *A. salina* in methanol extract of plant *P. methysticum* and E. suaveolens showed high mortality with low concentrations (100-1000 ppm) can reach 50-100% mortality after 24 hours of treatment. Methanol extracts of plants *X. novaguineense* and *M. aleuritoides* showed lower mortality rates at high concentration 1000 ppm only reached 33% after 24 hours of treatment.

Toxicity testing results of crude extracts showed the percentage of *A. salina* larvae mortality increased along with the increase in concentration of the extract. The results reveal that crude methanol plant extract of *P. methysticum*, and *E. suaveolens* showed that the compound contained therein are active and possess a high bioactivity, which means that at low concentrations has toxic and lethal larvae of *A. salina*.

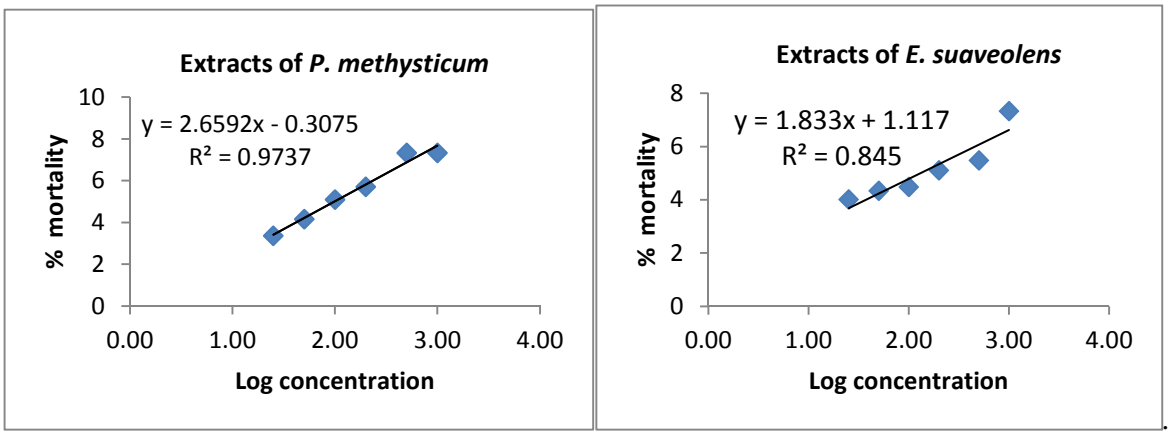
Table 3. The mortality rate and toxicity of methanol extract of plant leaves of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. aleuritoides*. using BSLT method

Plant extracts	Concentration (ppm)	Mortality ± SD (%)[a] 24 JSP	LC_{50} (ppm)	Description
P. methysticum	25	5,18 ± 2,99 a	99,05	toxic[b]
	50	19,77 ± 6,91 b		
	100	53,45 ± 5,17 c		
	200	75,86 ± 5,97 d		
	500	100,00 ± 0,00 e		
	1000	100,00 ± 0,00 e		
E. suaveolens	25	15,79 ± 10,53 a	131,34	toxic
	50	24,56 ± 6,08 ab		
	100	29,82 ± 8,04 b		
	200	54,39 ± 8,04 c		
	500	68,42 ± 5,26 d		
	1000	100,00 ± 0,00 e		
X. novaguineense	25	5,08 ± 2,936 a	5,372,86	mild toxic
	50	10,17 ± 7,767 a		
	100	11,86 ± 2,936 a		
	200	15,25 ± 10,585 ab		
	500	23,73 ± 5,085 bc		
	1000	32,20 ± 2,936 c		
M. aleuritoides	25	10,87 ± 8,774 a	6,710,94	mild toxic
	50	12,32 ± 10,781 a		
	100	19,89 ± 2,512 ab		
	200	19,89 ± 2,512 ab		
	500	27,52 ± 5,278 b		
	1000	32,90 ± 8,177 b		

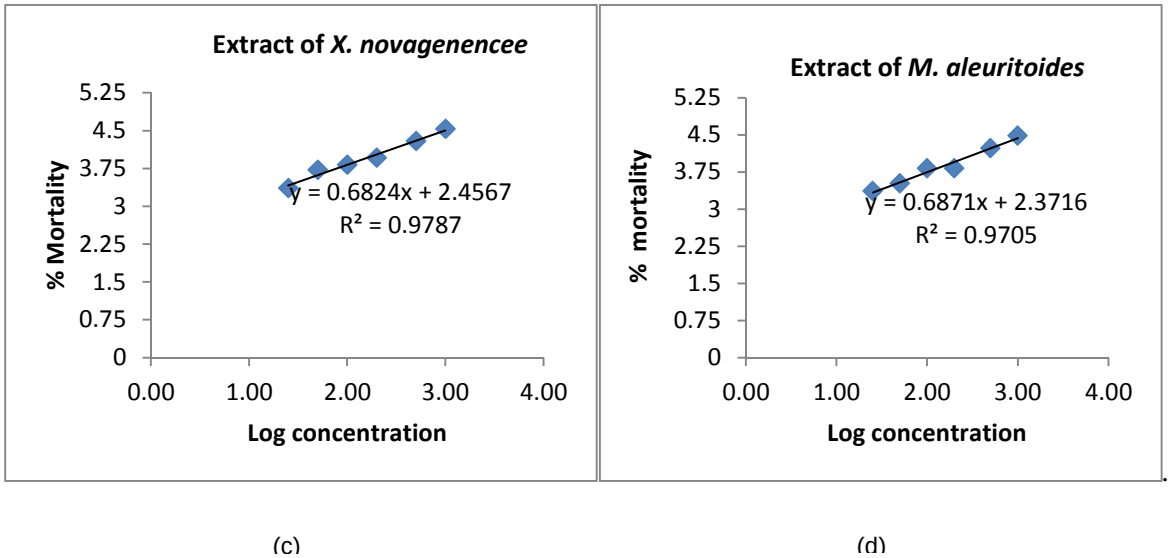
Description : [a] The average value (corrected) ± SD (standard deviation). Mean followed by the same letter are not significantly different by Duncan's multiple test (α = 0,05). JPS = Hours After Treatment. [b] Frank Lu toxicity criteria

The toxicity test to *A. salina* (Table 3), it is seen that the methanol extract of the leaves of *P. methysticum* and leaf extracts of *E. Suaveolens* have lower LC_{50} values are 99.05 ppm and 131.34 ppm. This suggests that *P. methysticum* extract and *E. suaveolens* has stronger toxicity activity of plant extracts *X. novaguineense* (LC_{50} = 5,372,86 ppm) and *M. aleuritoides* (LC_{50} = 6,710,94 ppm) are alleged to have mild toxicity properties. Benchmarks use of extracts of plant material as bioinsecticide is a high mortality rate [16]. The results of this test showed that the leaf extract of *P. methysticum* and *E. suaveolens* plant has the potential to be used as a bioinsecticide.

The Graph of regression analysis of methanol extracts from leaves of plants of *P. methysticum*, *E. suaveolens*, *X. novaguineense* and *M. aleuritoides* that presented in Fig 1.



(a) (b)



(c) (d)

Fig. 1. Graph relationship of log concentration of extract *P. methysticum* (a), *E. suaveolens* (b), *X. novaguineense* (c), *M. aleuritoides* (d) the mortality of larvae of *A. salina* response

From the results of probit analysis, LC_{50} values of methanol extract of *P. methysticum* (99.05 ppm) and *E. suaveolens* (131.34 ppm) lower than *X. novaguineense* (5.372,86 ppm) and *M. aleuritoides* (6.710, 94 ppm). The lower the LC_{50} value would indicate high toxicity effect, whereas the higher LC_{50} showed that the sample has a low toxicity. According to Mayer [9] extracts which have LC_{50} values > 1000 µg/ml are not categorized as toxic. The toxicological properties of the leaves of *P. methysticum* and *E. suaveolens* allegedly because compounds in it that alkaloids, flavonoids, saponins and tannins.

CONCLUSION

From the results of this study concluded that:
1. *P. methysticum* phytochemistry content and *E. suaveolens* plant are alkaloids, flavonoids, tannins and saponins, while plants *X. novaguineense* and *M. aleuritoides* contains flavonoids, tannins and saponins.
2. Toxicity tests using BSLT method methanol extract obtained LC_{50} values of plant of *P. methysticum* LC_{50} = 99.05 ppm and *E. suaveolens* LC_{50} = 131.34 ppm is more toxic than methanol extract of *X. novaguineense* that having LC_{50} = 5,372,86 ppm and M. aleuritoides LC_{50} = 6,710,94 ppm.

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