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BRIEF REPORT

Biological Activity and Phytochemical Analysis of Three Indonesian Medicinal Plants, *Murraya koenigii*, *Syzygium polyanthum* and *Zingiber purpurea*

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Abstract
Extracts of Indonesian medicinal plants, *Murraya koenigii*, *Syzygium polyanthum*, and *Zingiber purpurea* were investigated for their biological activity. The presence of phytochemicals, cytotoxicity, and antimicrobial and antioxidant activities were investigated. Parts of *M. koenigii*, *S. polyanthum*, and *Z. purpurea* were extracted with ethanol. The extracts were evaluated for antimicrobial activity using the disc diffusion method, while antioxidant activity was determined with a 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay. Cytotoxicity was investigated using the brine shrimp lethality test, and phytochemical screening was performed using a standard method. *M. koenigii* leaf extract exhibited the most activity in the test microorganism activity index (AI), 0.38–1.25, when compared with standard drugs. *S. polyanthum* ripened fruit displayed significant antioxidant activity (90%) in comparison to ascorbic acid (95%). *Z. purpurea* rhizome extract possessed the highest cytotoxic effect with a LC_{50} of 52 μg/mL. Phytochemical analysis revealed that carbohydrate, tannin, alkaloid, steroid, triterpenoid, and flavonoid were present in the extracts of *M. koenigii* leaves and twigs, *S. polyanthum* leaves and ripened and unripe fruits, and *Z. purpurea* rhizome, while saponin was only present in the *S. polyanthum* ripened fruit extract. Our work revealed that the *M. koenigii* leaves, *S. polyanthum* ripened fruit, and *Z. purpurea* rhizome extracts have potential as sources of new antimicrobial, antioxidant, and cytotoxic compounds, respectively.

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1. Introduction

Globally, research into biologically active natural products from plants has attracted many natural product chemists. Various plants have been examined for their biological activities and in some cases active substances have been isolated and identified. We believe in Indonesia most research activity into natural products is still limited to the inventory of folkloric information and utilization of various plants and trees, meaning that obtaining scientific proof for their biological activity is still challenging. *Murraya koenigii* (L) Spreng., *Syzygium polyanthum* (Wight) Walp., and *Zingiber purpurea* Rosc. are three popular medicinal plants used in Indonesia. The leaves of *M. koenigii* (Rutaceae), also known as Kare, are aromatic and commonly used in some Asian cooking. Antidiabetic and immunomodulatory effects of this plant have been reported [1,2]. The leaves of *S. polyanthum* (Myrtaceae), also known as Salam, is used as a culinary additive and also used for diabetes, diarrhea, and skin infection treatment [3,4]. The rhizome of *Z. purpurea* (Zingiberaceae), also known as Bangle, is used in folk medicine for the treatment of conditions such as inflammation, cough, and as a cleansing solution for skin diseases. In fact, the extract of *Z. purpurea* rhizome has been found to possess inflammatory activity [5]. Despite the extensive use by Indonesian people, there have been only limited attempts to explore the biological properties of these plants in relation to their medicinal uses. In our investigation into biological activities of Indonesian plants, we report here the antimicrobial and antioxidant potentials, cytotoxicity, and phytochemical analysis of the extracts from parts of *M. koenigii*, *S. polyanthum,* and *Z. purpurea*.

2. Materials and Methods

2.1. Plant materials and chemicals

M. koenigii (leaves and twigs), *S. polyanthum* (leaves, ripened and unripe fruits), and *Z. purpurea* (rhizome) were collected from Samarinda and Balikpapan, Indonesia, in May 2010. Voucher specimens were deposited in the Laboratory of Wood Chemistry, Faculty of Forestry, Mulawarman University, Indonesia under the accession code KK-1005-K009 and K010 for *M. koenigii*, KK-1005-S004 to S006 for *S. polyanthum*, and KK-1005-B004 for *Z. purpurea*. The plant materials were shade dried for 3 days and ground with a blender. DPPH (1,1-diphenyl-2-picrylhydrazyl) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). DMSO (dimethyl sulfoxide), sulfuric acid, hydrochloric acid, acetic anhydride, potassium iodide and peptone were purchased from Merck (Darmstadt, Germany). Ascorbic acid, 1-naphtol and bismuth (III) nitrate were obtained from Sigma (St. Louis, MO, USA). Nutrient agar was obtained from Difco (Detroit, MI, USA). Other chemicals were of HPLC grade or the highest purity commercially available.

2.2. Extraction

Ground *M. koenigii* leaves (679 g) and twigs (148 g); *S. polyanthum* ripened fruits (87 g), unripe fruits (237 g), and leaves (1395 g); and *Z. purpurea* rhizome (125 g) were extracted with 95% ethanol at room temperature with continuous shaking on a shaker (7400 Tübingen; Edmun Buchler, Germany) for 24 hours. This process was then repeated. Following filtration of the suspension through Whatman filter paper no. 2 (Maidstone, UK), the crude alcohol extract was rotoevaporated at 40ºC and put in a vacuum oven to near dryness to yield the extract of *M. koenigii* leaves (46 g, 6.8% DW) and twigs (8 g, 5.7% DW); *S. polyanthum* ripened fruits (13 g, 14.8% DW), unripe fruits (23 g, 9.7% DW), and leaves (182 g, 13% DW); and *Z. purpurea* rhizome (17 g, 13.8% DW).

2.3. Antimicrobial assay

Bacillus cereus, *Salmonella thypi*, *Tricophyton mentagrophytes*, and *Candida albicans* were used in all experiments. Nutrient agar and potato dextrose agar were used in antibacterial and antifungal assays, respectively. Plant extracts were dissolved in acetone to obtain a concentration of 40, 60, and 80 μg/10 μL, which was selected on the basis of our preliminary results showing the antimicrobial activity of plant extract tested simultaneously to correlate with the concentration of standard drugs used (10–20 μg/disk). Antimicrobial assays were conducted using the disc diffusion method as previously described by Kusuma et al [6]. Zones of inhibition around the discs were measured in mm. Activity index (AI) was calculated as the mean inhibition zone for test sample divided by the mean inhibition zone for the standard drug [7].

2.4. Antioxidant assay

The sample was first dissolved in DMSO and used at a 30 times dilution for the actual experiment. The DPPH radical scavenging method was performed as previously described by Arung et al [8]. UV absorption was measured on a Shimadzu UV-VIS 1240 spectrophotometer (Shimadzu Corp., Kyoto, Japan).

2.5. Cytotoxicity assay

The cytotoxicity assay was performed using the brine shrimp lethality test according to the method described in the literature [9]. Analysis of the data was performed by probit analysis on a Finney computer program to determine LC_{50} (Biostat 2008; AnalystSoft, Vancouver, Canada).

2.6. Phytochemical analysis

One gram of the plant ethanol extracts was dissolved in 100 mL ethanol and subjected to preliminary phytochemical screening following standard methods [10,11].

3. Results and Discussion

The results for antimicrobial tests of plant extracts are listed in Table 1. At 80 μg/disk, the extract of *M. koenigii* leaves and *S. polyantum* ripened fruit showed good activity with an AI of 1–1.25 to *Salmonella thypi* relative to erythromycin as a standard drug. The extracts showed moderate activity against *Bacillus cereus* with an AI of 0.31–0.62 in comparison to that of erythromycin. Good activity of the plant extracts was also observed against *Candida albicans* (AI 1–1.25 at 80 μg/disk) except with *S. polyanthum* leaves, suggesting the possibility that the extracts may be useful for the treatment of vaginal yeast infection, skin and diaper rash, and other *Candida* infection-caused diseases. The plant extracts had low to moderate activity against a dermatophyte, *T. mentagrophytes* (AI 0.25–0.41 at 80 μg/disk). Based on these results, higher concentrations of the extract seemed to increase the inhibition of *T. mentagrophytes* growth.

The results of antioxidant activity of *M. koenigii*, *S. polyanthum,* and *Z. purpurea* are presented in Figure 1. The extracts from *M. koenigii* leaves, *S. polyanthum* fruits, and *Z. purpurea* rhizome appeared to be more active than other extracts. The activities of these plant extracts were 86–90% at 100 ppm, which was comparable to that of the positive control ascorbic acid (95% activity).

Table 1 Antibacterial and antifungal activity of plant extracts

Extract tested (μg/disk)		Microorganisms tested							
		St		*Bc*		*Tm*		*Ca*	
		IZ*	AI†	IZ	AI	IZ	AI	IZ	AI
ML	40	12	1.0	13	0.50	11	0.34	8	0.73
	60	12	1.0	13	0.50	12	0.38	10	0.91
	80	15	1.25	12	0.46	12	0.38	11	1.00
MT	40	9	0.75	11	0.42	8	0.25	6	0.55
	60	10	0.83	12	0.46	9	0.28	9	0.82
	80	11	0.92	12	0.46	8	0.25	11	1.00
SRF	40	10	0.83	5	0.19	6	0.19	4	0.36
	60	11	0.92	8	0.31	11	0.34	8	0.73
	80	12	1.00	8	0.31	12	0.34	11	1.00
SUF	40	7	0.58	12	0.46	10	0.31	11	1.00
	60	8	0.67	13	0.50	11	0.34	12	1.09
	80	7	0.58	16	0.62	13	0.41	11	1.00
SL	40	6	0.50	5	0.19	8	0.25	0	0
	60	9	0.75	9	0.35	8	0.25	0	0
	80	11	0.92	11	0.42	9	0.28	0	0
ZR	40	8	0.67	6	0.23	8	0.25	0	0
	60	8	0.67	9	0.35	8	0.25	7	0.64
	80	10	0.83	9	0.35	8	0.25	11	1.00
ER		12	1.00	26	1.00	NT		NT	
MZ		NT		NT		32	1.00	NT	
CH		NT		NT		NT		11	1.00

*IZ's are presented as mean of triplicates and include the disc diameter (7 mm); †AI = IZ of test sample divided by the IZ of a standard drug. IZ = inhibition zone; AI = activity index; ML = *M. koenigii* leaves; MT = *M. koenigii* twigs; SRF = *S. polyanthum* ripened fruits; SUF = *S. polyanthum* unripe fruits; SL = *S. polyanthum* leaves; ZR = *Z. purpurea* rhizome; *St* = *S. thypi*; *Bc* = *B. cereus*; *Tm* = *T. mentagrophytes*; *Ca* = *C. albicans*; ER = erythromycin; MZ = miconazole; CH = chloramphenicol; NT = not tested.

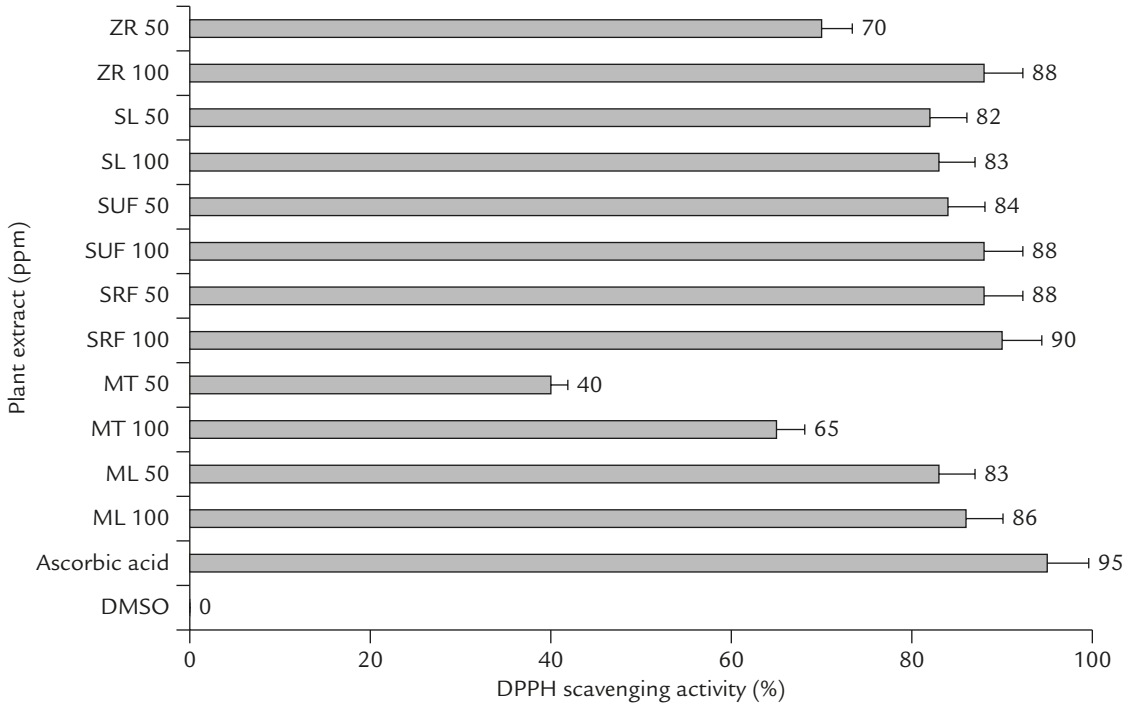

Figure 1 DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity of plant extracts. Ascorbic acid was 100 ppm. ML=*M. koenigii* leaves; MT=*M. koenigii* twigs; SRF=*S. polyanthum* ripened fruits; SUF=*S. polyanthum* unripe fruits; SL=*S. polyanthum* leaves; ZR=*Z. purpurea* rhizome.

The ripened fruit of *S. polyanthum* was the most active with 90% radical scavenging activity which was clearly more active than the unripe fruit. This observation may be related to the decomposition of chlorophyll into non fluorescing chlorophyll catabolites which is an effective antioxidant present during fruit ripening [12]. The search for antioxidants from natural sources has received much attention and efforts have been put into identifying compounds that can act as suitable antioxidants to replace synthetic ones. Further investigation into isolation of antioxidant compounds from the active extracts is in progress.

Plant extracts tested for cytotoxicity using the brine shrimp lethality test are presented in Table 2. *S. polyanthum* leaves and fruit extracts were shown to be inactive (LC_{50} >1000), while *M. koenigii* leaf and twig extracts and *Z. purpurea* rhizome extract showed significant activity (LC_{50} 197.84 μg/mL, 187.85 μg/mL, and 51.58 μg/mL, respectively). Therefore, the *M. koenigii* leaf and twig extracts and *Z. purpurea* rhizome have potential to be candidates for investigation as cytotoxic compounds. Cytotoxicity against brine shrimp shows strong correlation with cytotoxicity towards 9 KB (nasopharynx cancer), P388 (murine leukemia), and other cancer cells [13]. Therefore, cytotoxic properties of *M. koenigii* and *Z. purpurea* may open possibilities of these plant extracts possessing anticancer activity.

Table 2 Cytotoxicity of the plant extracts in the brine shrimp lethality test

Plant	Part used	LC_{50} (μg/mL)
Murraya koenigii	Leaves	197.84
	Twigs	187.85
Syzygium polyanthum	Leaves	>1000
	Ripened fruit	747.45
	Unripe fruit	>1000
Zingiber purpurea	Rhizome	51.58

Phytochemical screening of the plant extracts (Table 3) revealed that the crude extracts contained alkaloid, carbohydrate, tannin, alkaloid, steroid, triterpenoid, and flavonoid, while saponin was only present in *S. polyanthum*. The phytochemicals tested are known to exhibit medicinal activity and physiological activity. Flavonoids have been reported to possess antibacterial, antioxidant, anti-inflammatory, antiallergic, antimutagenic, and vasodilatory activity [14]. Saponins showed hypocholesterolemic and antidiabetic properties, while steroids and triterpenoids displayed analgesic properties [15–17]. The presence of biologically important phytochemicals in the *M. koenigii*, *S. polyanthum*, and *Z. purpurea* extracts, as tested for in this study, contribute to their medicinal value, and therefore, point to potential sources for useful drugs.

Table 3 Phytochemical analysis of the extracts of *Murraya koenigi*, *Syzygium Polyanthum*, and *Zingiber purpurea**

Phytochemicals	Plant extracts					
	ML	MT	SRF	SUF	SL	ZR
Saponin	−	−	+	−	−	−
Carbohydrate	+	+	+	+	+	+
Tannin	+	+	+	+	+	+
Alkaloid	+	+	+	+	+	+
Steroid	+	+	−	+	+	+
Triterpenoid	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+

*Plus sign indicates presence and minus sign indicates absence. ML=*M. koenigii* leaves; MT=*M. koenigii* twigs; SRF=*S. polyanthum* ripened fruits; SUF=*S. polyanthum* unripe fruits; SL=*S. polyanthum* leaves; ZR=*Z. purpurea* rhizome.

Further phytochemical and pharmacological investigations of the active compounds from *M. koenigii*, *S. polyanthum*, and *Z. purpurea* should be conducted given their diverse and extensive traditional uses and potential therapeutic applications.

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