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Evaluation of the phytochemical and biological properties of *Prismatomeris tetrandra* (Roxb.) K. Schum

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Abstract: The methanol extracts of the roots, stems, and leaves of *Prismatomeris tetrandra* were evaluated for their bioactivity on antiinflammatory, antioxidant, antimicrobial, and antidiabetic. The result showed that the stem showed the highest activity on lipoxygenase at 61.04 and antioxidant (DPPH Radical Scavenging Assay >100.0 + 0.0; Xanthine/xanthine oxidase Superoxide Scavenging System 74.6 + 2.6). The roots however performed the highest activity on insulinotropic assay at 1.8081 mg/ml) and antimicrobe against the growth of *Vancomycin-Resistant S. aureus*, *Vancomycin intermediate S. aureus*, and *Staphylococcus aureus* strains and able to kill the microbes. The MIC and MBC ranging between 0.625 to 2.50 mg/ml for all microbes. From the phytochemical screening and TLC profile, it can be concluded that different parts of this plant comprising of different chemical constituents. This might explain the differences in the bioactivity of different parts. The findings from this study can be used for further study in searching new active compound which can be used as a lead compound or this plant may be developed into a potential herbal product.

Keywords*: Prismatomeris tetrandra*, anti-inflammatory, antidiabetic, antioxidant, antimicrobe, phytochemical screening

INTRODUCTION

Plants have been used widely in traditional medicinal practices and are still applied in this modern living. Some of it becoming the beginning of natural drug discovery such as taxol which is derived from the bark of the Pacific yew tree (Taxus brevifolia) and used in the treatment of various cancer diseases nowadays. Research to obtain the pharmaceutical drugs from plant would take many years and it started from traditional claims and biological evaluation on the plant crude extract. Thus in taking the same attempt, the investigation was carried out on *Prismatomeris tetrandra* (Roxb.) K. Schum. *Prismatomeris tetrandra* was under the Rubiaceae family and well distributed in Peninsular Malaysia (Lemmens & Bunyapraphatsara, 2003). Since only very few reports on this plants' bioactivity, more biological evaluation should be performed on it. On our previous report, the anti-inflammatory properties of *Prismatomeris tetrandra* were investigated on the hyaluronidase and mouse ear edema induced with TPA inhibitory activity (Nor Hayati et al., 2017). In that study, ursolic acid was found as the bioactive compound responsible for the hyaluronidase inhibitory activity. *Prismatomeris tetrandra* activity towards several cancer cell lines

was also reported (Nor Hayati et al., 2019). In this study, different parts of this species (leaves, stem, and roots) were evaluated on several bioactivities including anti-inflammatory, antioxidant, antimicrobe, and antidiabetic. The objective of this study was to identify another biopotential of this species to be further developed for its therapeutic uses or upgraded into a potential herbal product.

MATERIAL AND METHODS

The roots, stems, and leaves were cut into smaller pieces and dried using an oven at 40oC. After that, it was ground. The sample then soaked into methanol for three days, filtered, and concentrated using a rotary evaporator at 45oC. The plants then soak again for another three days and the filtrate was combined. The process was repeated at least three times to get crude methanol extracts of leaves, roots, and stems.

*Lipoxygenase inhibition assay:*The assay was performed following the method by Nor Fariza, 2012. The amount of 160ul of 100 mM sodium phosphate buffer (pH 8.0) , 10µl test solution of the methanol extract, and 20 µl lipoxygenase enzyme solution were mix followed by incubation at 25 o C for 10 minutes. The reaction was then initiated by the addition of 10 µl linoleic acid as the substrate solution. The formation of (9Z,11E)-(13S)-13-hydroperoxyoctadeca-9,11-dienoate change in the absorbance at 234 nm. All test compounds and positive control were dissolved in DMSO. The reactions were performed in 96-well UV microplated in Tecan infinite M200 Microplate Reader (Tecan, Austria)

*DPPH Radical Scavenging system and xanthine oxidase Superoxide Scavenging activity :*The methods were following essay that was published by Markandan et al., 2016 for the anti-free radical assay.Whereas the scavenging activity evaluation for the extracts against superoxide free radical anions was perform by using the spectrophotometric method of Vimala et al., 2012.

Insulin Secretion assay: The assay was following the method by Chee et al., 2007. BRIN BD-11 cell lines were used. Cell growth and confluence were observed under an inverted microscope. Cells with exponential growth were seeded in culture plates at a concentration of 2.5 × 105 cells per well. The cells were transferred to a 24-wells culture plate, where they were incubated for 30 min with 1ml KRB buffer and plant extracts at 37 °C. After incubation aliquots were removed from each well and stored at -20 °C for insulin secretion assay. Insulin level was measured using a commercial insulin detection kit. The absorbance of aliquots was measured at 450 nm and insulin concentration (µg /l) was determined from the standard insulin concentration curve.

Antimicrobial assay: The methanol extracts were tested on five different strain microorganism gram-positive bacteria of *Vancomycin-Resistant S. aureus* (VRSA156), *Vancomycin Intermediate S. aureus* (VISA24), *Staphylococcus aureus* ATCC 25923 (MSSA) Sa2, *S. aureus* ATCC 33591 (MRSA/VSSA) Sa3, *S. aureus* ATCC 70069 (MRSA) Sa7. The gram-positive and negative bacteria were purchased from the American Type Culture Collection (ATCC) while VRSA156 and VISA24 were lab passage derived mutants from clinical MRSA isolate.
The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were following the method as described previously by Saiful et al., 2008 and Saiful et al.,2015 respectively. First well A was loaded with methanol extract stock solution (100 mg/ml) and topped up with respective broth. The mixture in well A was mixed thoroughly and transferring to the second well B to H containing respectively broth. The same procedure (well A) was repeated for well B to H to create a serial doubling dilution of the methanol extracts with concentrations ranging from 10 to 0.078 mg/ml.
A suspension containing 108 colony-forming units (CFU)/ml equivalent to McFarland standard tube no. 0.5 used (Vandepitte et al., 1991) were finally inoculated into the mixture to produce a

final volume of 100 µl. The positive control used were oxacillin and cyclohexamide whiles the DMSO-broth mixture (5%) was used as a negative control. The experiments were performed in triplicates and repeated twice. The lowest concentration which produces no visible growth or remains clear after observation through the macroscopic was taken as the MIC. The antimicrobial activity was categorized as strong (MIC ≤ 1000 µg/ml), moderate (999 µg/ml < MIC < 4900 µg/ml) and weak (MIC ≥ 5000 µg/ml) (Ibrahim et al., 2009). The MBC values were obtained by sub-culturing the contents of each negative well and from the positive control (broth with inoculum and without compound) of MIC determination, onto substance-free Mueller-Hinton agar (MHA) Petri dishes. The plates were incubated at 37oC for 24 hrs. The MBC was taken as the lowest concentration of the substance that results in more than 99.9 % reduction of the initial inoculum. Results were expressed as mean values of three independent determinations.

RESULTS

Table 1 summarizes the activity of three different parts of *Prismatomeris tetrandra* on four different bioactivities. Lipoxygenase (LOXs) are a family of lipid-peroxydizing enzymes that catalyzes the peroxidation of arachidonic acid and implicated in the pathogenesis of various inflammatory diseases including cancers. In this study, both stem and leaves showed almost similar activity with inhibition of 60.76 % and 60.76 % respectively. The results were classified as moderately active against lipoxygenase (41-70%) according to Lip et al., 2009. The extracts were also evaluated for their antioxidant activity by using two assays as shown in the table. The stems showed the highest and promising antioxidant activity on both assays used. It performed DPPH Radical Scavenging activity at >100.0 ± 0.0 and xanthine oxidase Superoxide Scavenging activity at 74.6 ± 2.6. The leaves and roots however performed the lowest activity for both assays. The DPPH Free Radical Scavenging Activity of the stem extract showed their effectiveness, prevention, interception, and repair mechanism against injury in a biological system (Long et al., 2001).Insulin is a hormone that controls the amount of glucose in the human bloodstream. In the antidiabetic activity investigation, the stem showed 1.7794 µg/L, which is an almost similar result to the roots 1.8081 µg/L. It suggests that both of the extracts have an insulinotropic effect on BRIN BD-11 cell lines and can be utilized as a modern candidate of antidiabetic agents targeting the escalation for insulin secretion from pancreatic beta cells (Nurshieren et al., 2018). The antimicrobial activity towards *Vancomycin-Resistant S. aureus*, *Vancomycin intermediate S. aureus*, and *Staphylococcus aureus* strains, the roots methanol extract showed the highest inhibitory activity towards the growth (MIC) and to kill the microbe (MBC) compared to the other parts. The inhibition ranging from 0.625 and 1.25 mg/ml for all the microbes, except for the MBC against *S. aureus* ATCC 33591 (MRSA/VSSA) were at 2.5 mg/ml. The different activities of different parts of *Prismatomeris tetrandra* could be influenced by their different chemical constituents. Table 2 summarise the phytochemical screening on the roots, stems, and leaves. Alkaloids were not detected on these plants. Triterpenes and steroids which were detected on the leaves could explain the anti-inflammatory activity of this part. Our previous bioassay-guided isolation study on the leaves part successfully isolated ursolic acid as the major and active compound of the leaves (Nor Hayati et al., 2017). Ursolic acid is a potent anti-inflammatory activity. It not only inhibits human leukocyte elastase (HLE), but also 5-lipoxygenase and cyclo-oxygenase activity (Najid 1992; safayhi 1997). A previous study on this plant reported that the chemical constituents of the roots mainly were the anthraquinone derivatives. The free aglycones (anthraquinone) are practically inactive. Therefore, further study needs to be conducted to identify the active compound responsible for the insulinotropic activity in the roots. The compound might be the same or different as in the stems. No report also on the chemical constituent of the stem that could explain the antidiabetic and good antioxidant activity of the stem part.

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Table 1: Anti-inflammatory, antioxidant, antidiabetic and antimicrobial activities of the different parts of *Prismatomeris tetrandra*

Extract	Anti inflammatory	Anti oxidant		Anti diabetic	anti microbe									
	Lipoxygenase (%)	DPPH Radical Scavenging Assay (%)	Xanthine /xanthine oxidase Superoxide Scavenging System (%)	Insulinotropic assay (ug/L)	Sa2 (MIC) mg/ml	Sa2 (MBC) mg/ml	Sa3 (MIC) mg/ml	Sa3 (MBC) mg/ml	Sa7 (MIC) mg/ml	Sa7 (MBC) mg/ml	VISA 24 (MBC) mg/ml	VISA 24 (MIC) mg/ml	VRSA 156 (MBC) mg/ml	VRSA156 (MIC) mg/ml
Leaves	60.76	24.7 ± 0.0	39.2 ± 1.2	1.1825	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
Stems	60.76	>100.0 ± 0.0	74.6 ± 2.6	1.7794	2.5	2.5	2.5	2.5	2.5	2.5	1.25	1.25	1.25	1.25
roots	55.53	25.8 ± 0.0	33.6 ± 1.55	1.8081	0.625	1.25	0.625	2.5	0.625	0.625	0.625	0.625	1.25	1.25
Positive control	99.13	100	100	2.6612	0. 312		0.312		0.625			2.500		1.250

Table 2: Phytochemical screening test on *Prismatomeris tetrandra*

Samples	Saponins	phenolic	Triterpenes	Alkaloids
Leaves	Detected	Detected (1+)	Triterpenes& steroids detected	Not detected
Stems	Detected	Detected (3+)	Triterpenes not detected	Not detected
Roots	Detected	Detected (3+)	Triterpenes not detected	Not detected

CONCLUSION

Three different parts of *Prismatomeris tetrandra* used in this study have different activities. It might be influenced by the different groups of compounds from different parts of this species. As a conclusion, this plant can be or have the potential to be developed into an herbal product with different application base on the activity. However, further study needs to be carried out for this plant to get more scientific information for more understanding of this plant.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

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