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Research Article

Evaluation of xanthine oxidase inhibitory, antioxidative activity of five selected Papua medicinal plants and correlation with phytochemical content

Septriyanto Dirgantara[1,2], Muhamad Insanu[1], Irda Fidrianny[1]

1 *Department of Pharmaceutical Biology, School of Pharmacy, Bandung Institute of Technology, Jl. Ganesa 10 Bandung, West Java 40132, Indonesia*
2 *Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Cenderawasih University, Jl. Kampwolker Waena Jayapura, Papua 99352, Indonesia*

Corresponding author: Muhamad Insanu (insanu@fa.itb.ac.id)

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Abstract

Some medicinal plants from Papua have been used traditionally as hyperuricemia and antioxidant agents for many ethnic in Papua Islands. The present study aims to evaluate inhibitory xanthine oxidase, antioxidative activities, total phenolic, and total flavonoid of five Papua selected medicinal plants, *Myrmecodia beccarii*, *Villebrunea rubescens*, *Breynia cernua*, *Bridelia* spp. and *Dodonaea viscosa*. Preliminary phytochemical screening revealed the presence of flavonoids and phenolic compounds with TLC-densitometric analysis, which is responsible for the biological activities of different plant extracts. Antioxidant was tested by 2,2- diphenyl-1- picrylhydrazyl (DPPH) and cupric ion antioxidant capacity (CUPRAC) methods and expressed as the Antioxidant Activity Index (AAI). Xanthine oxidase inhibitory (XOI) activity was evaluated by a spectrophotometer. The ethyl acetate of *Myrmecodia beccarii* extract (MEA) showed the highest antioxidative and XOI activity. The MEA extract from Papua may be developed as a new potential source of antioxidant and xanthine oxidase inhibitory agent.

Keywords

Papua, medicinal plants, xanthine oxidase, antioxidant, the phytochemical content

Introduction

Several Papua plants, such as *Myrmecodia beccarii* Hook. f, *Villebrunea rubescens* (Bl), *Breynia cernua* Muel. Arg, *Bridelia* spp., and *Dodonaea viscosa* Jacq. have long been used as medicinal plants by the Papuan people. Traditionally, local people in the Papua Province, Indonesia, have shared important information about medicinal plants in their communities to treat illness and various diseases, especially uric acid and gout therapies. The xanthine oxidase enzyme catalyzes the oxidation of xanthine to uric acid and hypoxanthine to xanthine. The oxidation produces superoxide radicals like hydrogen peroxide and reactive oxygen species (ROS) (Ramallo et al. 2006).

Xanthine oxidase inhibitors (XOI) will block the concluding step in uric acid biosynthesis that can reduce the plasma uric acid concentration level and are commonly applied for the treatment of gout (Finch and Kubler

2016). Natural products and antioxidant compounds from plants, vegetables, and fruits can inhibit the reaction of free radicals and xanthine oxidase inhibitors (Singh and Gupta 2018). Inhibition of XO can suppress the biosynthesis process of uric acid, which is one of the therapeutic approaches for treating gout, neuropathy, and kidney stones, which leads to hyperuricemia (Natsir et al. 2022).

The previous study only demonstrated the plants' ecology, taxonomy, and preliminary ethnopharmacological studies. Limited papers discussed the biological activities and the phytochemical content of Papua endemic medicinal plants. The potential active compound possessed by some plants from Papua, as well as their biological activity, is unknown. It is necessary to investigate XOI and antioxidative activities. This research aimed to evaluate XOI and antioxidative activities from five selected medicinal plants from Papua and to analyze their correlation with phytochemical content.

Materials and methods

Chemicals

Xanthine, xanthine oxidase, 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, gallic acid, cupric chloride, and neocuproine were obtained from Sigma-Aldrich Chemicals (St. Louise, MO, USA). The ascorbic acid was obtained from Merck (Darmstadt, Germany). Allopurinol was purchased from TCI (Tokyo, Japan), and all other reagents were analytical grade.

Sample collection

Fresh aerial parts of five selected medicinal plants, *Myrmecodia beccarii* Hook.f, *Villebrunea rubescens* (Bl), *Breynia cernua* Muel. Arg, *Bridelia* spp. and *Dodonaea viscosa* Jacq. were collected from five different locations in Papua Province-Indonesia and determined in the Herbarium of Research Center for Biology-Indonesian Institute of Sciences listed in Table 1. Samples were washed, dried, and milled into powder.

Table 1. Collection sample of selected medicinal plants from Papua region-Indonesia.

Scientific name	Local name	Sample location	Part(s) used	Family
Myrmecodia beccarii Hook.f	Sarang semut	Merauke	Tuber	Rubiaceae
Villebrunea rubescens (Bl)	Daun jilat	Serui	Leaves	Urticaceae
Breynia cernua Muel. Arg	Katuk hutan	Jayapura	Leaves	Euphorbiaceae
Dodonaea viscosa Jacq.	Dollu	Lanny Jaya	Leaves	Sapindaceae
Bridelia spp.	Sampare	Biak	Leaves	Phyllanthaceae

Extract preparation

Extraction was conducted using Soxhlet with increasing polarity solvent, ranging from nonpolar (n-hexane), semipolar (ethyl acetate), and polar solvent (96% ethanol). Crude drug powder 100 grams of five medicinal plants from Papua, *Myrmecodia beccarii* Hook.f, *Villebrunea rubescens* (Bl), *Breynia cernua* Muel. Arg, *Bridelia* spp., and *Dodonaea viscosa* Jacq. were extracted with n-hexane, and the filtrate and residue were then separated. The residue was dried and extracted with ethyl acetate. Finally, the residue was extracted using 96% ethanol. All extracts were evaporated using a rotary vacuum evaporator with a temperature of 50 °C to produce fifteen extracts. There were five n-hexane extracts (*M. beccarii* (MBH), *V. rubescens* (VRH), *B. cernua* (BCH *Bridelia* spp. (BSH), and n-hexane extract of *D. viscosa* (DVH)); five ethyl acetate extract (*M.beccarii* (MEA), *V. rubescens* (VEA), *B.cernua* (BEA), *Bridelia* spp. (BSE) and ethyl acetate extract of *D. viscosa* (DEA)) and five ethanol extract (*M.beccarii* (MET); *V. rubescens* (VET), *B. cernua* (BCE), *Bridelia* spp. (BET) and ethanolic extract of *D. viscosa* (DET)). The phytochemical test was performed on crude drugs and extracts using the method described in Farnsworth and Sarker's modified method (Farnsworth 1966; Sarker 2013).

Total phenolic content (TPC)

The total phenolic content was evaluated with a Folin-Ciolcalteu reagent adapted to Pourmorad's method (Pourmorad et al. 2006). Each extract of 0.5 mL was piped into 5 mL Folin-Ciolcalteu reagent 10% and 4 mL of sodium carbonate 1M and incubated the mixtures for 15 minutes. The absorbance was measured at wavelength 765 nm. For each extract, the analysis was performed in triplicate. The results were reported as g gallic acid equivalents (GAE) per 100 g extract (g GAE/100 g) according to the calibration curve of gallic acid 25–150 µg/mL as standard.

Total flavonoid content (TFC)

The total content of flavonoids was measured using an adapted method from Chang (Chang et al. 2002). Each extract of 0.5 mL was piped into 0.1 mL aluminum chloride 10%, 0.1 mL sodium acetate 1M and 2.8 mL distilled water. The mixture was diluted with 1.5 mL ethanol and incubated for 30 minutes at wavelength 415 nm, and the absorbance was read. For each extract, the analysis was carried out in triplicate. The results were presented as g equivalents of quercetin (QE) per 100 g extract (g QE /100 g) according to the calibration curve of quercetin 5–100 µg/mL as standard.

In vitro antioxidant activities by DPPH assay

Determination of the AAI (Antioxidant Activity Index) based on Blois's method (Blois 1958) with some modification was applied to determine antioxidative activity by DPPH. Each extract was prepared in various concentrations and mixed with the DPPH solution of 50 µg/mL (volume 1:1). The DPPH solution was dissolved in methanol. The absorbance was evaluated at 517 nm by UV-vis spectro-

photometry after 30 minutes of incubation. The standard was ascorbic acid. Analysis was investigated in triplicate. The purple color of the DPPH solution will be shifted when the antioxidant scavenges the free radical, so an inhibitory concentration of 50% (IC_{50}) can be determined from the sample. A calibration curve was plotted between % (the percentage) of DPPH scavenging activity versus concentration to obtain IC_{50}. After that, the AAI of each extract was determined by dividing the final concentration of DPPH with IC_{50} at each extract (Scherer and Godoy 2009).

In vitro antioxidant activities by CUPRAC assay

The AAI (Antioxidant Activity Index) CUPRAC was determined based on Apak's method (Apak et al. 2013). Several extract concentrations were added to the CUPRAC solution of 100 µg/mL (volume 1:1). After incubation for 30 minutes, the absorbance was read at 450 nm. The CUPRAC solution was prepared by mixing $CuCl_2$ in distilled water and neocuproine in ethanol, and then the mixture was dissolved in ammonium acetate buffer pH 7. Ascorbic acid was applied as standard. Analysis was performed in tri-replication. If the sample acts as an antioxidant agent, Cu (II) will reduce to Cu (I). Neocuproine's function is to make a chromophore with Cu (I) and show a yellow color, and then the exhibitory concentration of 50% (EC_{50}) was obtained from the calibration curve. Afterward, the AAI of each extract can be calculated by dividing the final concentration of CUPRAC with EC_{50}.

Xanthine oxidase inhibitory activity assay

The inhibitory activity of xanthine oxidase was measured by spectrophotometer in 96-well plates (Corning, UV-Transparent Clear Microplates) below the aerobic conditions, following the method reported by Owen and Duong with some minor modification (Owen and John 1999; Duong et al. 2017). The extracts were dissolved in dimethyl sulfoxide (DMSO) and diluted with phosphate buffer pH 7.5. The final DMSO concentration was not higher than 0.5%. Allopurinol was used as a standard. The test solution consisted of 50 µl of the sample of extract (100 µg/mL), 69 µL of phosphate buffer, 15 µL of freshly made solution for the enzyme (0.2 unit/mL xanthine oxidase in phosphate buffer, then 66 µL of substrate solution (0.15 mM of xanthine in phosphate buffer). The test solution was incubated at 25 °C for 15 minutes. The absorbance was measured at 15 minutes using a microplate reader (Tecan infinite M200 pro) at λ 290 nm. In the same procedure, a blank was prepared, but the enzyme was substituted by phosphate buffer. The analysis for allopurinol and each extract was performed in triplicate. The inhibitory activity of xanthine oxidase was determined using the formula: $I\% = [(A - B)/A] \times 100$, where A = absorbance of enzyme xanthine oxidase without test extract – blank of A (absorbance without XO and test extract), B = absorbance of the test extract – blank of B (absorbance without XO).

TLC-densitometric analysis

TLC-densitometric studies were carried out using the standard method by Wagner (Wagner et.al 1996). 25 mg extract was dissolved in 0.5 mL methanol and centrifuged at 3000 rpm for 5 minutes. These solutions were used as test solutions for phenolics and flavonoid. 2 µL of test solutions and standart solution (caffeic acid, quercetin, and naringenin) were loaded as 5 mm band length in the aluminium silica gel 60 F_{254} TLC plates (5 × 10 cm). The linear ascending development was eluted in a saturated mode condition with twin through chamber room using the eluent system toluene: ethyl acetate: formic acid (4:2:0.2). The plate was dried at room temperature before being scanned using densitometry (CAMAG TLC Scanner 3 with winCATS 1.4.2 software) at 254 nm and 366 nm UV light.

Statistical analysis

The results were the means ± SD of at least three independent tests, using MS Excel Software to evaluate the IC_{50} and EC_{50} values. Analysis of the statistic (one-way ANOVA – *post hoc Tukey*) was carried out by SPSS 23. Using Pearson's method, correlations were made between the total phenolic and flavonoid content with antioxidant and xanthine oxidase inhibitory activities.

Results and discussion

Extraction

In the present investigation, antioxidative activity, total phenolic and flavonoid content, xanthine oxidase inhibitory activities of five medicinal plants from Papua, *M. beccarii*, *V. rubescens*, *B. cernua*, *Bridelia* spp., and *D. viscosa* were evaluated. Extraction was carried out by Soxhlet with different polarity solvents, three solvents n-hexane, ethyl acetate, and ethanol. Three other polarities solvents, such as n-hexane, ethyl acetate, and ethanol, were used to separate based on the polarity of compounds in crude drugs. n-hexane and ethyl acetate were selected solvents. Therefore, the nonpolar compounds will be mainly extracted in n-hexane and semipolar compounds in ethyl acetate. Meanwhile, ethanol will find most of the polar compounds. The extracts' preliminary qualitative investigation revealed phytochemical compounds such as alkaloids, flavonoids, triterpenoids/steroids, quinone, saponin, tannin, and phenols. The phytochemical screening results were carried out from each extract of the Soxhlet extraction method using increasing polarity solvents from five plants *M. beccarii*, *V. rubescens*, *B. cernua*, *Bridelia* spp., and *D. viscosa* were displayed in Table 2. Based on phytochemical screening results, all crude drugs and extracts detected flavonoids, phenol, tannin, saponin, and steroid/triterpenoid. Meanwhile, alkaloid was not seen in *M. beccarii*, and quinone was

not detected in *V. rubescens*, *B. cernua*, and *D. viscosa*. Secondary metabolite compounds from plants that contain flavonoid and phenolic compounds have the potential to be antioxidant and xanthine oxidase inhibitor agents from natural resources (Ryu et al. 2012; Petkova et al. 2017).

Table 2. Phytochemical screening of crude drugs and various extracts.

Sample		Phytochemical screening						
		Alkaloid	Flavonoid	Steroid/ Triterpenoid	Quinone	Saponin	Tannin	Phenols
MB	CD	-	+	+	+	+	+	+
	MBH	-	-	+	-	-	-	-
	MEA	-	+	+	+	+	+	+
	MET	-	+	-	-	+	+	+
VR	CD	+	+	+	-	-	+	+
	VRH	-	+	+	-	-	-	-
	VEA	+	+	+	-	-	-	+
	VET	-	-	-	-	-	+	-
BC	CD	+	+	+	-	+	+	+
	BCH	-	-	+	-	-	-	-
	BEA	+	+	-	-	-	+	+
	BCE	-	+	-	-	+	+	+
BS	CD	+	+	+	+	+	+	+
	BSH	-	-	+	-	-	-	-
	BSE	+	+	+	+	-	-	-
	BET	-	+	-	-	+	+	+
DV	CD	+	+	+	-	+	+	+
	DVH	-	-	+	-	-	-	-
	DEA	+	+	-	-	-	+	+
	DET	+	+	-	-	+	+	+

CD = crude drug, n-hexane extract of *M. beccarii* (MBH), ethyl acetate extract (MEA), ethanol extract (MET); n-hexane extract of *V. rubescens* (VRH), ethyl acetate extract (VEA), ethanol extract (VET); n-hexane extract of *B. cernua* (BCH), ethyl acetate extract (BEA), ethanol extract (BCE); n-hexane extract of *D. viscosa* (DVH), ethyl acetate extract (DEA), ethanol extract (DET); n-hexane extract of *Bridelia* spp. (BSH), ethyl acetate extract (BSE), and ethanol extract (BET); (+) = detected; (-) = not detected.

Extract density

Density is a crucial assay to compare its activity and phytochemistry compound levels. Higher density will result in stronger activity and phytochemical content. The measurement of the concentrated extract was carried out using a 1% extract solution in a pycnometer. In this study, the density of each extract showed values in the range of 0.661–0.895 g/mL.

In determining the TPC in each extract based on Table 3, gallic acid was used as standard, and a linear regression equation of gallic acid was applied y = 0.0068× - 0.0577, R^2 = 0.9872. The TPC in all Papua medicinal plant extracts presented different results from 2.40 to 9.01 g GAE 100/ g. Ethanolic extract of *B. cernua* (BCE) showed the highest TPC (9.01 g GAE/100 g). Meanwhile, quercetin was used as standard for determining TFC with the liner regression y = 0.0105× – 0.0078, R^2 = 0.9907. The TFC in all Papua medicinal plant extracts presented various results from 0.89 to 8.03 g QE/100 g. Ethyl acetate extract of *Bridelia* spp. gave the highest TFC (8.03 g QE/100 g). Phenolic compounds generally have antioxidative activity due to the ability to donate electrons or as chelating metals. The antioxidative activity of flavonoids was influenced by the degree of hydroxylation and other substituents found in flavonoids (Heim et al. 2002). Flavonoid compounds will give high antioxidant power through the transfer hydrogen mechanism when it has ortho di-OH in C3'-C4', C2-C3 double bond, OH in C3, and oxo in C4. Ortho di-OH in C3'-C4' had the highest effect on antioxidative activity. Flavonoids with OH substitution on rings A and B belong to the phenolic group (Fidrianny et al. 2013). Phenolic compounds and flavonoids have xanthine oxidase inhibitory activity like apigenin, luteolin, quercetin, myricetin, morin, and kaempferol (Nagao et al. 1999; Dong et al. 2013). A high antioxidant capacity in a flavonoid will be presented when the flavonoid has a hydroxyl group in C-3′-C-4′, OH at C-3, oxo function at C-4, double bond at C-2 and C-3 (Rice-Evans et al. 1996).

Table 3. The yield, density of extract, TPC, and TFC in various extracts.

Sample	Yield (%)	The density of extract 1% (g/mL)	TPC g GAE/100 g	TFC g QE/100 g
MBH	3.41	0.661	5.37 ± 0.16^{a}	1.22 ± 0.13^{a}
MEA	9.12	0.780	7.92 ± 0.24^{b}	2.80 ± 0.04^{b}
MET	19.15	0.895	4.41 ± 0.16^{c}	1.67 ± 0.03^{c}
VRH	6.75	0.665	7.14 ± 0.18^{d}	2.93 ± 0.02^{b}
VEA	5.29	0.673	2.40 ± 0.29^{e}	2.40 ± 0.02^{d}
VET	8.84	0.784	3.43 ± 0.07^{f}	2.63 ± 0.04^{b}
BCH	6.35	0.758	4.08 ± 0.18^{c}	2.30 ± 0.01^{d}
BEA	11.62	0.857	7.09 ± 0.11^{g}	3.08 ± 0.01^{e}
BCE	22.53	0.893	9.01 ± 0.23^{h}	0.99 ± 0.02^{f}
BSH	2.85	0.697	6.07 ± 0.18^{i}	2.49 ± 0.01^{d}
BSE	3.79	0.722	8.42 ± 0.17^{b}	8.03 ± 0.02^{g}
BET	30.13	0.898	6.92 ± 0.18^{j}	3.30 ± 0.02^{h}
DVH	9.27	0.767	4.53 ± 0.07^{k}	1.52 ± 0.02^{c}
DEA	17.17	0.866	5.77 ± 0.06^{l}	4.98 ± 0.05^{i}
DET	22.93	0.894	3.12 ± 0.17^{m}	0.89 ± 0.02^{f}

n-hexane extract of *M. beccarii* (MBH), ethyl acetate extract (MEA), ethanol extract (MET); n-hexane extract of *V. rubescens* (VRH), ethyl acetate extract (VEA), ethanol extract (VET); n-hexane extract of *B. cernua* (BCH), ethyl acetate extract (BEA), ethanol extract (BCE); n-hexane extract of *D. viscosa* (DVH), ethyl acetate extract (DEA), ethanol extract (DET); n-hexane extract of *Bridelia* spp. (BSH), ethyl acetate extract (BSE), and ethanol extract (BET); the reported values are mean ± SD (n=3). Different letters in the same column indicate a significant difference ($p<0.05$).

Based on Table 4, the higher antioxidative activity was demonstrated by lower IC_{50} DPPH and EC_{50} CUPRAC or higher AAI of DPPH and AAI CUPRAC. The ethyl acetate extract of *M. beccarii* (MEA) with an AAI value of 3.22 for AAI DPPH and 4.27 for AAI CUPRAC were classified in the category of very strong antioxidants (AAI > 2). Meanwhile, the AAI value for standard ascorbic acid was 10.20 for DPPH and 11.92 for CUPRAC (Scherer and Godoy 2009).

A recent study (Dirgantara et al. 2013) confirmed that the methanol extract of *M. beccarii* showed antioxidative activity with IC_{50} 8.18 µg/mL. Many species of the Myrmecodia genus showed potential antioxidative activity with the DPPH method. The free radical scavenging activity (antioxidative activity) of the ethanol extract of hypocotyl *Myrmecodia pendens* was evaluated using DPPH radical. The IC_{50} value occurred at 96.21 µg/mL of extract and contained total phenol and flavonoid contents 330.61 mg GAE/ g and 63.28 mg QE/ g of dry extract, respectively

(Engida et al. 2013). The ethyl acetate fraction of this extract contained procyanidin B1 dimer (3.236 mg/g dry sample) and rosmarinic acid (20.688 mg/g dry sample) significant free radical scavenging capacities with IC_{50} values of 27.59 µg/mL and 35.80 µg/mL, respectively (Engida et al. 2015). Ethanolic extract of stem bark, leaves, and tuber of *Myrmecodia tuberosa* showed antioxidative activity with 95.17%, 94.55%, and 93.42% DPPH scavenging activities, respectively (Rasemi et al. 2014). The previous study investigated ethyl acetate fractions of the hypocotyl of *Myrmecodia platytyrea* and showed antioxidative activity with IC_{50} 21.57 µg/mL (Mohamad Haris et al. 2016). Polyphenols and flavonoids of ethanol and ethyl acetate extracts expressed the highest antioxidative activity compared to dichloromethane and methanol extracts of *M. platytyrea* with high-performance thin-layer chromatography (HPTLC)-DPPH bioautographic method (Agatonovic-Kustrin and Morton 2018b; Agatonovic-Kustrin et al. 2018a).

Based on Table 5, it can be seen there were significant and positive correlation between TPC in VR extract with AAI DPPH (r = 0.636, p<0.05); TPC and TFC in MB, BC and BS extract with AAI DPPH (r = 0.880, p<0.01; r = 0.986, p<0.01; r = 0.889, p<0.01; r = 0.850, p<0.01; r = 0.976, p<0.01; r = 0.959, p<0.01, respectively). Meanwhile, TPC and TFC in MB extracts with AAI CUPRAC (r = 0.952, p<0.01; r = 0.961, p<0.01, respectively). Based on this result, it can be concluded that phenolic and flavonoid compounds in MB, BC, and BS extract were the main compound for antioxidative activity using DPPH and CUPRAC assays.

The correlation between two antioxidant testing DPPH and CUPRAC methods has also been exposed in Table 6. The different antioxidant capacities assays will give different results. Two methods with different antioxidant mechanisms might give no linear result. Pearson's correlation could analyze whether the AAI of each method gave a linear result or not. DPPH is a combination of hydrogen transfer and electron transfer. Meanwhile, CUPRAC electron transfer only. Two methods will give linear results when they show positive and linear correlation. The result demonstrated that AAI DPPH of MB and BC extract positively and significantly correlated with their AAI CUPRAC (r = 0.973, p<0.01; 0.883, p<0.01, respectively). TPC in ethyl acetate of *B. cernua* extracts (BEA) 7.14 ± 0.18 g GAE 100/g was in line with ethyl acetate of *M. beccarii* extract (MEA) 7.92 ± 0.24 g GAE 100/ g, but AAI DPPH BEA (0.28) was lower than AAI MEA (3.22). It can be suggested that most phenolic constituents in MEA showed higher potent antioxidative activity, which had many hydroxyl substituents in their structure. In the CUPRAC assay, a sample will act as an antioxidant when it has a reduction potential smaller than Cu^{2+}/Cu+ (1.59V). TFC in n-hexane of *V. rubescens* extract (VRH) 2.93 ± 0.02 g QE/100 g was in line with ethyl acetate of *M. beccarii* extract (MEA) 2.80 ± 0.04 g QE 100/g, but AAI CUPRAC MEA (4.27) was higher than AAI VRH (0.22). So, the MEA can be categorized as a remarkably potent antioxidant, while VRH is a poor antioxidant. It can be suggested that most of the flavonoids in MEA had reduction potentials smaller than 1.59 V, while only a few flavonoids in VRH were lower than 1.59 V.

Table 4. AAI value of DPPH and CUPRAC in various extracts.

Sample	IC_{50} DPPH (µg/mL)	AAI DPPH	EC_{50} CUPRAC (µg/mL)	AAI CUPRAC
MBH	182.80 ± 1.79[a]	0.14 ± 0.01[a]	152.39 ± 1.32[a]	0.33 ± 0.01[a]
MEA	7.82 ± 0.65[b]	3.22 ± 0.26[b]	11.73 ± 0.45[b]	4.27 ± 0.16[b]
MET	29.93 ± 0.64[c]	0.84 ± 0.02[c]	109.66 ± 0.98[c]	0.46 ± 0.01[c]
VRH	174.17 ± 1.64[d]	0.14 ± 0.01[d]	223.90 ± 1.72[d]	0.22 ± 0.01[d]
VEA	187.08 ± 2.66[f]	0.13 ± 0.01[f]	184.83 ± 1.07[e]	0.27 ± 0.01[e]
VET	212.23 ± 1.87[d]	0.12 ± 0.01[d]	148.29 ± 0.89[f]	0.34 ± 0.01[f]
BCH	137.16 ± 1.68[e]	0.18 ± 0.01[e]	80.63 ± 2.18[g]	0.62 ± 0.02[g]
BEA	88.93 ± 1.56[g]	0.28 ± 0.01[g]	136.94 ± 0.57[h]	0.37 ± 0.01[h]
BCE	33.25 ± 0.83[c]	0.75 ± 0.02[c]	44.27 ± 0.28[i]	1.13 ± 0.01[i]
BSH	171.98 ± 3.76[d]	0.15 ± 0.01[d]	103.69 ± 0.40[j]	0.48 ± 0.01[j]
BSE	11.86 ± 1.91[b]	2.17 ± 0.37[b]	36.04 ± 0.27[k]	1.39 ± 0.01[k]
BET	34.69 ± 0.99[c]	0.72 ± 0.02[c]	25.71 ± 0.57[l]	1.95 ± 0.05[j]
DVH	135.89 ± 1.06[e]	0.18 ± 0.01[e]	60.71 ± 0.24[m]	0.83 ± 0.01[m]
DEA	35.28 ± 0.70[i]	0.71 ± 0.01[i]	22.05 ± 0.19[n]	2.27 ± 0.02[n]
DET	16.51 ± 0.96[h]	1.52 ± 0.09[h]	27.75 ± 0.05[l]	1.80 ± 0.01[l]
Ascorbic Acid	2.45 ± 0.02	10.20 ± 0.10	4.19 ± 0.10	11.92 ± 0.29

n-hexane extract of *M. beccarii* (MBH), ethyl acetate extract (MEA), ethanol extract (MET); n-hexane extract of *V. rubescens* (VRH), ethyl acetate extract (VEA), ethanol extract (VET); n-hexane extract of *B. cernua* (BCH), ethyl acetate extract (BEA), ethanol extract (BCE); n-hexane extract of *D. viscosa* (DVH), ethyl acetate extract (DEA), ethanol extract (DET); n-hexane extract of *Bridelia* spp. (BSH), ethyl acetate extract (BSE), and ethanol extract (BET); the reported values are mean ± SD (n=3). Different letters in the same column indicate a significant difference (p<0.05).

Table 5. Correlation of TPC and TFC with antioxidant parameter.

Antioxidant Parameter	Pearson's Correlation Coefficient (r)	
	TPC	TFC
AAI DPPH MB	0.880**	0.986**
AAI DPPH VR	0.636*	0.497[ns]
AAI DPPH BC	0.889**	0.850**
AAI DPPH BS	0.976**	0.959**
AAI DPPH DV	-0.618*	-0.261[ns]
AAI CUPRAC MB	0.952**	0.961**
AAI CUPRAC VR	-0.669*	-0.471[ns]
AAI CUPRAC BC	0.557[ns]	-0.998**
AAI CUPRAC BS	0.473[ns]	0.269[ns]
AAI CUPRAC DV	0.284[ns]	0.648*

MB = *M. beccarii*, VR = *V. rubescens*, BC = *B. cernua*, BS = *Bridelia* spp., DV = *D. viscosa*; **= significant at p<0.01, *= significant at p<0.05, ns = not significant.

Table 6. Correlation Pearson of DPPH and CUPRAC methods.

Antioxidant Parameter	Pearson's Correlation Coefficient (r)				
	AAI CUPRAC MB	AAI CUPRAC VR	AAI CUPRAC BC	AAI CUPRAC BS	AAI CUPRAC DV
AAI DPPH MB	0.973**				
AAI DPPH VR		-0.932**			
AAI DPPH BC			0.883**		
AAI DPPH BS				0.390[ns]	
AAI DPPH DV					0.563[ns]

MB = *M. beccarii*, VR = *V. rubescens*, BC = *B. cernua*, BS = *Bridelia* spp., DV = *D. viscosa*; **= significant at p<0.01, ns = not significant

Based on Fig. 1, the ethyl acetate extract with a concentration of 100 µg/mL of *M. beccarii* extract (MEA) showed the highest potential XOI activity with a percentage of inhibition of 66.42 ± 2.89%. Meanwhile,

allopurinol, a standard with a concentration of five µg/mL, showed 80.84 ± 0.88%. Based on this research, ethyl acetate extract of *M. beccarii* extract (MEA) showed the highest potential xanthine oxidase inhibitory and antioxidative activity in line with phytochemical total phenolic content. The previous study by Simanjuntak et al. (2010) investigated the other species, *M. pendens* showed the highest inhibition at 61.99% from n-butanol fraction, and the isolated compound showed the xanthine oxidase inhibitory activity at 79.77%.

TLC-densitometric analysis showed that ethyl acetate extract of *M. beccarii* extract (MEA) revealed the presence of various phytochemical contents, as illustrated the Fig. 2 and Table 7. The chromatograms were obtained upon scanning at UV 254 nm, and peak tables were generated. All phytochemical compound peaks were identified based on Rf values of MEA extract compared with standard caffeic acid, naringenin, and quercetin. MEA extract exerted eight prominent bands, of which peak number 7 was identified as caffeic acid compared with the Rf values and blue fluorescent band color of the caffeic acid standard.

The research results showed that each extract of the Papua medicinal plants plant gave a different pattern of xanthine oxidase inhibitory and antioxidative activity depending on the solvent used. This can be due to the other chemical content in every fifteen extracts. According to the result, it can be presumed that the total phenolic content of extracts contributed to its antioxidative activity and xanthine oxidase inhibitory activity.

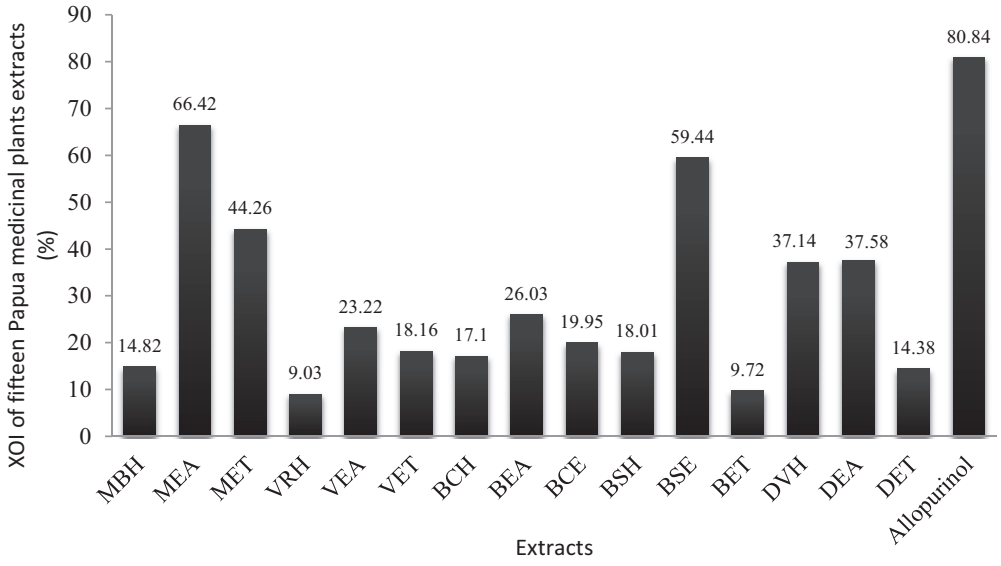

Figure 1. Percentage of XOI fifteen Papua medicinal plant extracts.

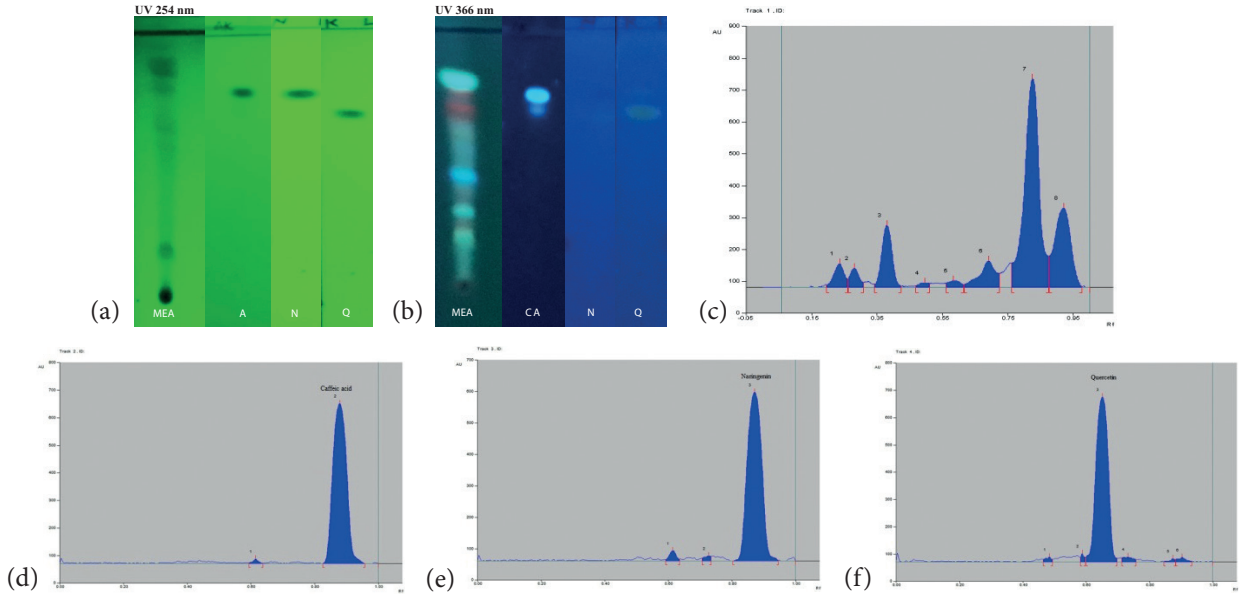

Figure 2. TLC profile of MEA extract with standard caffeic acid (CA), naringenin (N) and quercetine (Q). **a**) UV light 254 nm, **b**) UV light 366 nm, **c**) densitogram MEA extract, **d**) caffeic acid, **e**) naringenin, **f**) quercetin.

Table 7. Phytochemical compounds peak identified in TLC MEA extract.

Peak	Maximum Rf	Area	% Area	Assigned Substance
1	0,23	2165,4	4,08	unknown
2	0,28	1505,7	2,83	unknown
3	0,38	5546,8	10,83	unknown
4	0,50	406,8	0,77	unknown
5	0,58	715,3	1,35	unknown
6	0,69	3703,4	6,97	unknown
7	0,82	27374,2	51,52	Caffeic acid
8	0,92	11380,8	21,65	unknown

Conclusion

The present results showed that all fifteen Papua medicinal plant extracts had a variety of xanthine oxidase inhibitory, antioxidative activity, and phytochemical content. Total phenolic and flavonoid content of ethyl acetate extract of *M. beccarii* (MEA) gave a significant and positive linear correlation with AAI DPPH and CUPRAC assays and xanthine oxidase inhibitory activity. Phenolic and flavonoid compounds in *M. beccarii* extract significantly contributed to the antioxidative activity and XOI. The ethyl acetate of *M. beccarii* (MEA) originated from Papua and may be developed as a potential source of new antioxidant and xanthine oxidase inhibitory agent.

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