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Original Article

Phytochemical properties and antioxidant activities of the leaf extracts of *Boenninghausenia albiflora* (Hook.) Rchb. ex. Meisn. (Rutaceae)

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Abstract

Boenninghausenia albiflora is a well-known plant for being used in various traditional treatments. The plant is also reported to contain an essential oil which has a distinctive and pungent aroma. The present study aimed at investigating extraction yields, phytochemical compounds and antioxidants, and potential uses of *B. albiflora leaves* extracted with acetone solvent. Bioactive compounds of the leaf extract of *B. albiflora* were identified by GC-MS analysis while the antioxidant assay was measured by DPPH method. The extract obtained from acetone contained 30 compounds including coumarin group, 2-Isoprenyl-2,3-dihydro-furo [3,2-G] chromen-7-one (22.89% w/v) as the highest percentage compound. Other coumarins such as suberosin and dehydrogeijerin were also identified in this study. The IC_{50} values of the leaves extract of *B. albiflora* was 194.3 ppm which was higher than propyl gallate. Thus indicating *B. albiflora* as potential sources of anti-cancer, anti-inflammatory and antioxidant.

Keywords: Anti-cancer, anti-inflammation, antioxidant, *Boenninghausenia albiflora*, chemical compounds.

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Introduction

Boenninghausenia albiflora (Hook.) Rchb. ex Meisn. is a monotypic genus belong to Rutaceae family. This deciduous shrub is thought to be native of the Indian region and distributed to the Southeast and Southwest of China (Takhtajan, 1986). This species can grow in tropical to sub-tropical regions with an altitude of 500 - 3000 meter above the sea level. *B. albiflora* is found growing in Bhutan, Nepal, Pakistan, Kashmir, India, Indonesia, Philippines, Myanmar, Thailand, Laos, North Vietnam, China and Japan (Flora of China, 2016).

Traditionally *B. albiflora* has been used for several medicinal purposes, such as a wound remedy, treatment of bleeding, and infection (Medicinal Herbs, 2016; Tirta et al., 2015). The leaf squash of *B. albiflora* can also be utilized to treat skin diseases. While all parts of this species can be used to relieve headaches by placing them under a pillow during sleeping (Medicinal Herbs, 2016). Whereas the boiled root is used to treat malaria (Tirta et al., 2015).

The information about phytochemical compounds of *B. albiflora* essential oils and their potential has been reported several times. Padalia et al. (2012) had revealed about chemical composition of leaf and root essential oils of *B. albiflora* from Northern India. They found that the leaf essential oil *B. albiflora* contained dominant compounds β-myrcene, (Z)-β-guaiene, (Z)-β-ocimene and β-caryophyllene, whereas the root essential oil contained bicyclogermacrene, α-terpinyl acetate, geijerene and β-copaene-4α-ol. It has also been reported that *B. albiflora* leaves contain essential oils with a distinctive and pungent aroma which potential use as anti-microbial, antioxidant, anti-inflammatory, anti-proliferation, natural fragrance, and aromatherapy (Wibawa et al, 2019). While Mehmood et al. (2017) found that the essential oil of *B. Albiflora* has potentials as an hepatoprotective and an antidiabetic agent base on in vivo study.

Although several studies of the *B. albiflora* essential oil have been carried out, but the studies of the chemical properties and potential of the *B. albiflora* extract are still very rarely reported in the scientific journal. Therefore, this topic will be very interesting to study. So the aim of this study was to observe the chemical content of acetone extract of *B. albiflora* from Bedugul Bali and to examine its chemical constituents and determine its potential for human uses.

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Method

Plant Material

The mature leaves of *B. albiflora* was obtained from the Plant Collection of Bali Botanic Garden (Fig. 1). This species was collected from the Mount Rinjani National Park, Lombok Island, West Nusa Tenggara in 2014 and planted in the Bali Botanic Garden in the same year. Determination of plant species was carried out by Ida Bagus

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Ketut Arinasa as a taxonomist of Bali Botanic Garden Herbarium.

Preparation of Plant Extract

The fresh and mature leaves of *B. albiflora* were harvested and separated from the stem and branches. The leaves were dried by avoiding direct sunlight for several days (more or less 5 days or until the leaf completely dried) in the room temperature and avoided from the direct sunlight. A total of 100 g dried leaves was mashed with a blender and extracted by maceration method with acetone solvent (100 g material in 1000 ml methanol) for 72 h. The extract solution was filtered using the Whatman filter paper and evaporated by using Vacuum Rotary Evaporator IKA–RV10. The evaporated extract was analyzed with GC-MS to determine the phytochemical compounds.

GC-MS Analysis

The acetone extract of *B. albiflora* was analyzed by GC-MS equipment Merk Shimadzu QP 2010. A total of 1 µL extract was injected to column RTx 5 MS (60 m x 0.25 mm) with film thickness 0.25 µm, oven temperature was programmed between 50-280 °C at a rate of temperature rise 5 °C/min, the carrier gas used was Helium with pressure 101 kPa, total flow rate 46.5 mL/min, column flow rate 0.85 mL/min, cleaning flow rate 3.0 mL/min and split ratio 1:50. The chemical components were determined by comparing the spectral data and retention time of GC-MS analysis result with the recorded data in the computer library WILEY7.LIB.

Figure 1. Flowers and Leaves of *B. albiflora*

2,2-Diphenyl-1-picrylhydrazil (DPPH) assay

The DPPH assay was conducted using the method from Widowati et al. (2017) with some modification to determine antioxidant activity. A series of concentrations of the leaves extract of *B. Albiflora* (50, 75, 100, 200, and 300 ppm) were used to make a linearity regression of DPPH scavenging activity. Briefly, 1 mL of the leaves extracts of *B. albiflora* were put in a bottle glass, then they were added with 4 mL DPPH (Sigma Aldrich D9132, USA 40ppm in methanol). These mixtures were incubated in dark place for 30 min, and measured the absorbances by spectrophotometer Thermo Scientific Genesys 30 with 517 nm wavelength. The radical scavenging activity was calculated using the formula:

$$\text{Scavenging Activity \%} = (Ac - As) / Ac \times 100$$

Ac: control absorbance (without sample)
As: sample absorbance

The linearity regression graph of scavenging activity was made by Microsoft excel. The IC50 (Half concentration of inhibition) value was measured by the regression formula. IC50 is the concentration required to result in a 50% antioxidant activity. The smaller the IC50 value means that the content of antioxidant compounds contained in plant extracts is higher and the antioxidant activity is higher.

Result

Based on the GC-MS analysis, acetone extract of *B. albiflora* leaves contained 29 identified chemical compounds. The identified chemical compounds and the chromatogram of GC-MS analysis in acetone extract of *B. Albiflora* were performed in table 1 and figure 2. the major components of the *B. albiflora* leaves extract were 2-Isoprenyl-2,3-dihydro-furo [3,2-G] Chromen-7-one (22.89% w / v).

The GCMS analysis result also showed that acetone extract of *B. albiflora* leaves contained several compounds with a relative concentration more than 2% (Figure 3.). They were formamide (3.27%), Acetid Acid (2.02%), 2,3-Dihidro-benzofuran (4.41%), 9,12,15-Octadecatrien-1-ol (2.11%), 2-Isopropenyl-2,3-Dihydro-Furo[3,2-G]Chromen-7-One (22.89%), Suberosin (2.37%), 2H,6H-Benzo[1,2-b:5,4-b']dipyran-2,6-dione, 7,8-dihydro-8,8-dimethyl- (5.45%), Bravelin (2.49%), Dehydrogeijerin (4.57%), 3-hydrocarbonyl-5-hydroxy-7- (5.37%), 9-Dehydro-1-Methyl Estrone Methyl Ether (3.43%) and 17.beta.,19-Diacetoxy-4,4-dimethyl-13.alpha.-androsta-5,7-dien-3-one (5.87%) (sorted by retention time).

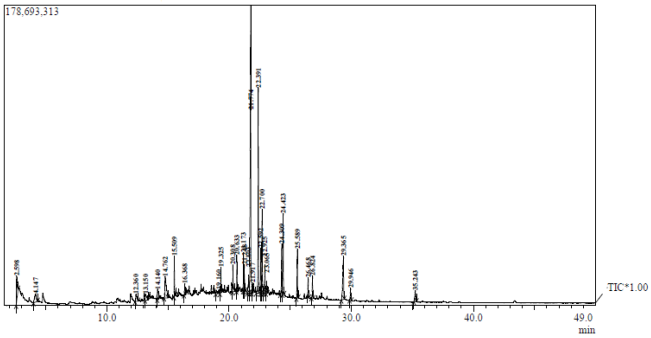

Figure 2. The Chromatogram of GC-MS analysis in acetone extract of *B. Albiflora*

Table 1. The GC-MS analysis results in acetone extract of *B. albiflora* leaves

Peak#	R.Time	Area	Conc%	Name
1	2.598	76304731	3.27	Formamide (CAS) Methanamide
2	4.147	46967133	2.02	Acetic acid (CAS) Ethylic acid
3	12.360	29646258	1.27	2-Cyclopenten-1-one, 2-hydroxy-3-methyl- (CAS) Corylon
4	13.150	31493640	1.35	Phenol, 3-methyl- (CAS) m-Cresol
5	14.140	27433184	1.18	Phenol, 3-ethyl- (CAS) m-Ethylphenol
6	14.762	102715001	4.41	2,3-Dihydro-Benzofuran
7	15.509	46115806	1.98	7-Cyano(15n)-Cycloheptatriene
8	16.368	23175873	0.99	D-Glucose, 4-O-[3-acetyl-1-(trimethylsilyl)-1H-indolyl]-2,3,5,6-tetrakis-O-(trimethylsilyl
9	19.160	22953253	0.98	1,2-Benzenediol, 4-(2-amino-1-hydroxyethyl)-, (R)- (CAS) .alpha.-(aminomethyl)
10	19.325	31449700	1.35	1-Hexadecyne (CAS)
11	20.308	45540764	1.95	Hexadecanoic acid (CAS) Palmitic acid
12	20.633	45119964	1.94	4-Cyanophenyl 4-Propylbenzoate
13	21.173	37612686	1.61	Seselin
14	21.248	28243944	1.21	2-Propenoic acid, 3-[4-[(3-methyl-1-butenyl)oxy]phenyl]-, methyl ester (CAS)
15	21.603	49184085	2.11	9,12,15-Octadecatrien-1-ol (CAS) octadeca-9,12,15-trien-1-ol
16	21.774	533368939	22.89	2-isopropenyl-2,3-dihydro-furo[3,2-g]chromen-7-one
17	21.917	27538821	1.18	Hexadecanamide (CAS) Amide 16
18	22.391	282971684	12.14	-
19	22.592	55303249	2.37	Suberosin
20	22.700	127081671	5.45	2H,6H-Benzo[1,2-b:5,4-b']dipyran-2,6-dione, 7,8-dihydro-8,8-dimethyl- (CAS)
21	22.925	58107110	2.49	Bravelin
22	23.065	24988623	1.07	Isopimpinellin
23	24.309	106417774	4.57	Dehydrogeijerin
24	24.423	125121538	5.37	3-hydroxycarbonyl-5-hydroxy-7-pentyl-9-oxa-10,10-dimethyl-1,2,4a,10a-tetrahydrophenanthrane
25	25.589	79950075	3.43	9-dehydro-1-methyl estrone methyl ether
26	26.468	28393768	1.22	Triacontane (CAS) n-Triacontane
27	26.824	37704863	1.62	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, acetate (CAS) Menthol acetate
28	29.365	136747775	5.87	17.beta.,19-Diacetoxy-4,4-dimethyl-13.alpha.-androsta-5,7-dien-3-one
29	29.946	26650801	1.14	Pentatriacontane (CAS) n-Pentatriacontane
30	35.243	36207845	1.55	Pentatriacontane (CAS) n-Pentatriacontane
		2330510558	**100.00**	

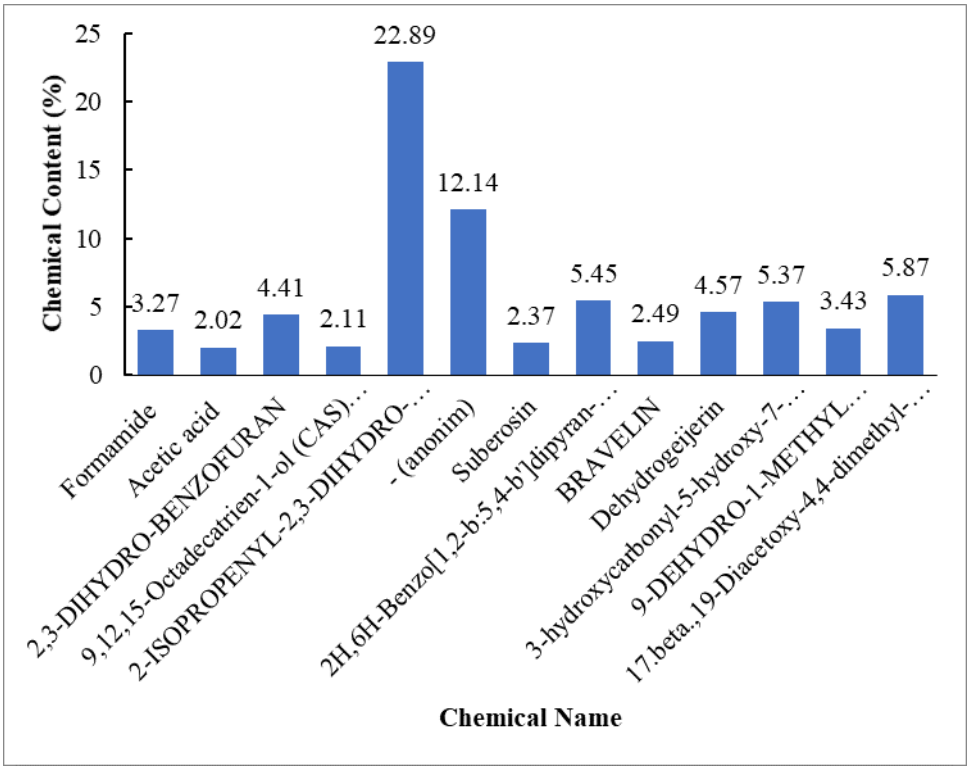

Figure 3. The chemical contents of acetone extract of *B. albiflora* with relative concentration more than 2%

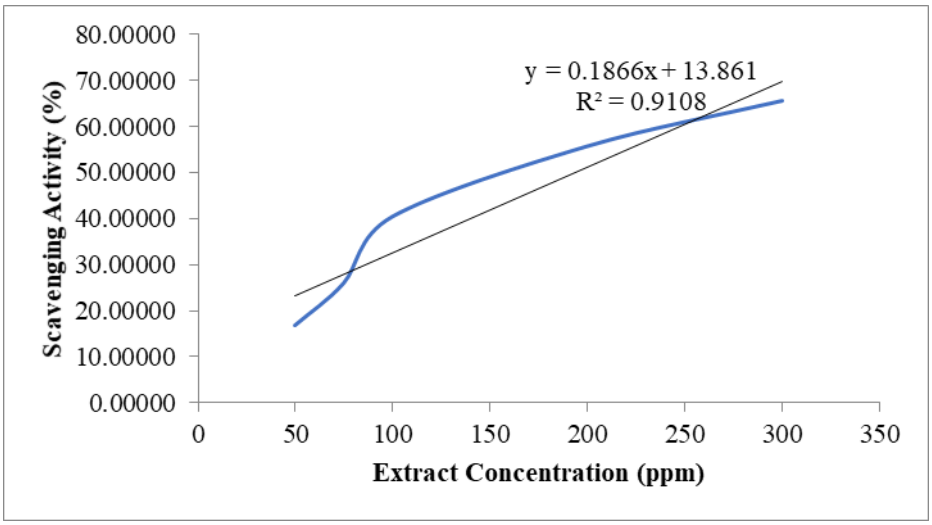

Figure 4. The regression graph of DPPH scavenging activity percentage of the leaves extract of *B. albiflora*

2,2-Diphenyl-1-picrylhydrazil (DPPH) assay

The values of the percentage of the free radical scavenging activity of leaves extract of *B. albiflora* in different concentration was exhibited in table 2. and its regression graph was showed in figure 4. DPPH assay showed that the IC50 value of the leaves extract of *B. albiflora* was 194.30 ppm.

Table 2. the percentage of DPPH scavenging activity of leaves extract of *B. albiflora* in different concentration

ppm	% Scavenging activity
50	16.73640
75	25.94142
100	40.44630
194.30	**50 (IC 50)**
200	55.78801
300	65.69038

Discussion

The results exhibited that the dominant contents of the leaves extract of *B. albiflora* was 2-Isoprenyl-2,3-dihydro-furo [3,2-G] Chromen-7-one (22.89% w/v), a chemical compound belong to the coumarin group. It has been reported that coumarin group posses various pharmacology activities such as antioxidant, Anti-inflammatory, anti-bacterial, anti-hyperglycemic, anti-adipogenic and anticancer (Tavares da Silva et al., 2019), insecticides (insect poisons), antiplatelet (drugs to prevent the formation of blood clots, useful for treating heart disease), antiplasmodial (antimalarial), antifeedant (food inhibitors, in insects) and growth inhibitory properties (Bhattacharya et al., 1989; Co et al., 1990; Escoubas et al., 1992; Gaur, 1999; Oketch-Rabah et al., 2000). The majority of coumarin compounds can be extracted using acetone solvents. *B. albiflora* belong to Rutaceae family and majority of Rutaceae family members are known for their ability to produce a variety of coumarin natural products (Barik et al., 1983; Chen et al., 1995; Ishii et al., 1993; Reisch and Achenbach, 1989). According to Sharma et al. (2006) ethanol extracts of *B. albiflora* showed insecticide activity against several forest plant pests. Coumarin compounds isolated from Murraya exotica were also reported to have insecticide activity against a number of pest species (Barik & Kundu, 1987). In addition, this extract also contains other identified coumarin compounds such as Suberosin (2.37 % w/v) and Dehydrogeijerin (4.57 % w/v). Both of these compounds are known to have anti-cancer and anti-inflammatory activity (Chen et al., 2007; Deok et al., 2012).

This study also revealed that the acetone extract of *B. albiflora* leaves contained 12 chemical components with relative concentration more than 2% and some other compounds which relative concentrations are less than 2% but have potential use for industry and pharmacology. Such as Formamide. This compound is also known as methanamide.it is present in small amounts in acetone extract of *B. albiflora* leaves (3.27% w/v). This compound is an amide derived from formic acid. Formamide is a clear liquid that is soluble in water and has an ammonia-like odor. Formamide is a chemical raw material for the manufacture of sulfa drugs, other medicines, herbicides, pesticides and manufacturing of hydroxylic acid. Formamide is used as a softener for paper and fiber and as a solvent for many ionic compounds and also as resins and plasticizers (Hohn, 1999).

Acetic acid is contained in an amount of 2.02% w/v in the acetone extract of *B. albiflora* leaves. This compound has many functions, but most are used as chemical reagents, fungicides, herbicides, microbiosides, pH adjusters and solvents in various industries, such as food, agriculture, cleaning and cosmetics. Injection of acetic acid into tumor has been used to treat cancer since the 1800s (Shibata, 1998). Acetic acid is used as part of cervical cancer screening in many regions in developing countries (Domgue et al., 2015). Acetic acid is an effective antiseptic with a broad spectrum of activity against streptococci, staphylococci, pseudomonas, enterococci, etc. (Madhusudhan, 2015; Ryssel et al., 2009). Acetic acid is

also used to treat skin infections caused by pseudomonas strains that are antibiotics resistant (Nagoba et al., 2013).

B. albiflora leaf extract also contained Pentatriacontane as much as 2.69%. Pasdaran et al. (2018) reported that the Pentatriacontane compounds contained in *Scrophularia amplexicaulis* and *Scrophularia oxysepala* had antibacterial activity against *Streptococcus aureus.* Menthol acetate was another compound found in this extract (1.62% w/v). Previous study revealed that menthol acetate had effect of reducing mild pain caused by muscle strains or sprains, backaches, bruises, and cramps. Menthol was a topical analgesic that worked by temporarily relieving mild pain. Menthol also had antiseptic properties that could inhibit germs (Sumardjo, 2006). Triakontanol also existed in the amount of 1.22 % w/v. Ries (1985) reported that Triacontanol had the potential to increase the citrus production and increase the ration of sugar to acid in citrus plant, but the work mechanism had not been fully known. Triacontanol could also increase *Camellia sinensis* tea production (Karssen et al., 1992).

Hexadecanoic acid or Palmitic acid presented in small amount (1.95 % w/v). This chemical compound is a saturated fatty acid commonly found in palm oil. Palmitic acid is often used in the beauty industry as a component of soaps and other cleaning products because it has surfactant properties. Palmitic acid is also an excellent emollient that has potential to maintain the natural moisture and to smooth the skin (Sumardjo, 2006).

1-Hexadecyne and m-Cresol were found in the acetone extract of *B. albiflora* leaves as much as 1.35 % w/v. According to Selvi and Basker (2012) 1-Hexadecyne had antibacterial properties. m-Cresol (1.35%) is a precursor of various active compounds such as pesticides (phenitrothion and fenthion), synthetic vitamin E (Mukhopadhyay, 2004), and antiseptics (such as amylmetacresol) (Morrell, 2003). As for the other compounds contained in this extract have not been studied in biological activity.

Antioxidant Assay

By comparing the IC50 Value of the leaves extract of *B. albiflora* (194.30 ppm) with the other members in Rutaceae family, the IC 50 value of *B. albiflora* can be categorized as a high category. Momeni et al. (2010) reported that IC50 value of *Vepris heterophylla* R. Letouzey was 204.69 ppm. They claimed that this species might be a good source of an antioxidant because it exhibited high free radical scavenging activities. While Kassim et al. (2013) found that the IC50 of methanol extract of Bark of *Melicope glabra* (Rutaceae) was found 810.02 ppm. The IC50 of both species in Rutaceae Family was lower than the IC50 of *B. albiflora* leaves extract. It means, *B. abliflora* leaf contains antioxidants in quite high amounts and has potential to be a new source of the exogenous antioxidant. The exogenous antioxidant was an important component to ameliorate the damage in human body caused by oxidative stress. Antioxidant compounds can inhibite he initiation or propagation of oxidative chain reaction, playing a rule as free radical scavengers, quenchers of singlet oxygen and reducing agents (Tan et al., 2018).

In conclusion, acetone extract of *B. albiflora* leaves was dominated by coumarin compounds. *B. albiflora* leaf was suspected having potential as an anti-cancer and anti-inflammatory agents because it contained Suberosin and Dehidrogeijerin compounds. *B. albiflora* leaf extract also had various other potential as an antioxidant, insecticide, antimalarial agent and preventing the blood clots.

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