Original Research Article

Phytochemical Screening of *Peronema Canescens* Jack

Ku Fareez Ku Halim, Kathleen J. Jalani, Hannis Fadzillah Mohsin & Ibtisam Abdul Wahab*

Department of Pharmaceutical Pharmacology and Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia.

Abstract

Peronema canescens Jack, locally known as sungkai, is used as medicinal plant because it contains chemical compounds such as peronemins, catechol, quinic acid, isovanillic acid and guaiacol. The study was conducted to determine the chromatographic profile of the *P. canescens* leaves extract *via* liquid chromatography. It was found that the optimised mobile phase for thin layer chromatography (TLC) of *P. canescens* leaves was hexane: ethyl acetate: isopropanol (7:3:0.3). In addition, high performance liquid chromatography (HPLC) chromatograms (λ = 210 and 254 nm) of the methanol extract, run in 0.1% formic acid and acetonitrile, provided eight acceptable peaks.

Keywords: extraction, chromatography, *Peronema canescens* Jack

Corresponding author
Ibtisam Abdul Wahab
Level 11, FF1 Building, Faculty of Pharmacy,
UiTM Puncak Alam, Bandar Puncak Alam, 42300,
Selangor, Malaysia
ibtisam@uitm.edu.my

Received 22 July 2019; accepted 27 February 2020

Available online: 31 March 2020
https://doi.org/10.24191/IJPNaCS.v1.02

1.0 Introduction

Peronema canescens Jack is a timber tree that grows to a height of 15 m with a girth of 60 cm in the rain forests of Indonesia and Malaysia (1). It is classified under Verbenaceae family. It has long history of being used as a medicinal herb by our ancestors either by using its leaves or barks. *P. canescens* was used to treat diseases, infection and inflammation. Ningsih and Ibrahim (2) found that the leaves of *P. canescens* can inhibit the growth of *Staphylococcus aureus, Bacillus subtilis,* and *Streptococcus mutans*. Matsuura *et al*. (3) discovered that *P. canescens* extracts played a role as anti-parasitic. It contains peronemins which inhibited the growth of *Babesia gibsoni* and *Babesia canis* with moderate anti-babesial activity. Earlier, Kitagawa *et al*. (4) discovered that peronemins can exhibit inhibitory activity, especially through the action of peronemin C_1 and A_3 against *Plasmodium falciparum.* Yani and Putranto (5) conducted a study and found that *P. canescens* extract of young leaves at the dosage of 0.5625 mg/kg (w/w) was able to lower the temperature of the body of mice by 29% which was faster than paracetamol (26%).

Kitagawa *et al*. (4) conducted the extraction by using acetone on the air-dried leaves of *P. canescens*. The samples were subjected to silica gel column chromatography for isolation procedure. It was found that the plant has unique compounds; 7 clerodane-type diterpenoids known as Peronemin A_2, A_3, B_1, B_2, B_3, C_1, and D_1. The barks of *P. canescens* were analysed by gas chromatography and showed several dominant compounds such as catechol, quinic acid, isovanillic acid, guaiacol, hydroquinone, genkwanin, and benzoic acid (6).

However, there is a lack of screening and phytochemical study of *P. canescens* that can further enhance its role in pharmaceutical field (7). Thus, this study was aimed to extract the *P. canescens* leaves by using chloroform, hexane, and methanol, and to screen the chemical compounds through thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Therefore, it is hoped that this research would increase the awareness of benefits that *P. canescens* could provide.

2.0 Materials and Methods

2.1 Chemicals

All chemical reagents were analytical grade solvents: chloroform, methanol as well as n-hexane, which were purchased from Merck (Germany). Pre-coated silica gel 60 F254 sheets (Merck, Darmstadt, Germany) and TLC twin trough development chambers were used.

2.2 Samples

P. canescens leaves were collected from Kuala Berang, Terengganu, Malaysia, in February 2019. The leaves were air-dried in dark room. Then, they were ground by using grinder.

2.3 Extraction (Maceration)

70 g of *P. canescens* leaves were weighed and placed inside a glass container. 800 ml of chloroform was measured by using measuring cylinder and was added (until the solvent filled the container). Then, the samples were covered with aluminium foil. The first two steps were repeated by using different solvents; methanol and hexane. The samples were left for 1 week. The solvents were filtered by using filter paper to remove the residues. Each solvent was evaporated to obtain the concentrated extracts by using rotary evaporator. The concentrated solvent extracts were placed inside small glass jar.

2.4 Thin layer chromatography (TLC)

A TLC plate with measurement of 5 cm x 10 cm was obtained. The plate is an aluminium sheet which is coated with a thin layer of a solid adsorbent (silica). One cm

from baseline was drawn by using pencil. Then, the solvent front with a measurement of 7.5 cm and the locations for the extracts to be dropped were drawn. Three drops of each extract; methanol, chloroform and hexane, were dropped on each designated place on the silica plate. Mobile phase e.g. hexane: ethyl acetate: chloroform (6:4:2) was prepared and transferred into a TLC developing chamber. The chamber was shaken and allowed to stand for a while, to saturate the chamber with solvent vapours. Then, the TLC plate was placed inside TLC chamber. It was placed in a way that the plate was soaked in a shallow pool of the solvent mixture. Then, the TLC chamber was closed by using its lid. The mobile phase would develop on the silica TLC plate as it slowly rose up the plate by capillary action. The plate was removed after the mobile phase had reached the solvent front. The silica plate was observed at daylight, and under UV with $\lambda = 254$ nm and 365 nm. The spots were observed and circled by using pencil. The Rf value was calculated and tabulated into Table 1.

2.5 Detection by spraying reagent

Sulphuric anisaldehyde spray was prepared by adding together 0.5 ml *p*-anisaldehyde in 50 ml glacial acetic acid and 1 ml 97% sulphuric acid. Then, it was transferred inside a glass sprayer. This reagent was used to visualize the spots on the TLC plates that were not seen under UV. The Rf value of the spots was recorded in Table 1.

2.6 Preparative TLC

The preparative TLC plate was used. A thin line was drawn with pencil on the plate; 2 cm from bottom, 1.5 cm from both side left and right. Continuous drops of the chloroform extract were placed at point of origin 6 times (on the thin line from bottom). A mobile phase, consisting of hexane: ethyl acetate: isopropanol (7:3:0.3) was prepared and placed inside the preparative TLC chamber (total volume of mobile phase = 103 ml). Then, the chamber was shaken and allowed to stand for a while, to saturate the TLC chamber with solvent vapours. Next, the preparative TLC plate was placed inside the chamber and closed with a lid. The plate would be removed when the mobile phase had reached the solvent front. After the mobile phase had reached the desired solvent front, the plate was taken out from the jar. After that, the plate was observed under daylight and UV light ($\lambda = 254$ nm and 365 nm). The developed bands were circled with a pencil. Then, the preparative TLC plate was covered at the middle between left and right thin line with an aluminium foil. Then, the exposed area of left and right of the plate was sprayed with sulphuric anisaldehyde. The aluminium foil was removed and the bands that produced colour were circled with a pencil. Then, each band was scratched and dissolved into chloroform, which was the same solvent that was used to extract the leaves. The scratched bands were filtered with cotton wool and transferred into separate vial. After that, the vials were covered with aluminium foil.

2.8 High performance liquid chromatography (HPLC)

The methanol extract of *P. canescens* leaves was prepared by filtering the extract with membrane filter (pore size = 0.45 µm). The extract was then diluted to appropriate concentration. The mobile phase that was made up of 0.1% formic acid and acetonitrile, was prepared. The vials containing the methanol extract and acetonitrile (blank) were added to the autosampler tray. The HPLC system was set up by using the parameters (8) (Table 1). The system was run and the chromatographic data was recorded.

3.0 Results

The *P. canescens* leaves extract were applied to the TLC plate by using a thin end of the spotter. It was placed in the concentrated extract; then the liquid would

Table 1: The HPLC settings.

Test solution	1 mL of methanol extract of *P. canescens* leaves was diluted with 5 mL of methanol and filtered.		
Column	Agilent C18 (5 µm, 4.6 mm i.d. x 150 mm)		
Mobile phase	Run time (min)	A – 0.1% formic acid	B - Acetonitrile
	0.00	90.0	10.0
	50.00	60.0	40.0
	55.00	60.0	40.0
	60.00	90.0	10.0
	65.00	90.0	10.0
Injection Volume	20 µL		
Flow Rate	1 mL/ min		
Column Temperature	Ambient		
Detection	PDA 254 nm		

rise by the capillary forces. The end of the spotter touched the plate briefly at the start line. The solvent was allowed to evaporate and the sample was spotted at the same place again. This could produce a concentrated extract on a small spot. Then, the plate was placed in a TLC chamber containing the mobile phase. Based on Figure 1, there were five mobile phases that showed remarkable and interesting results; Hex: EA: $CHCl_3$ (6:4:2), Hex: EA: IsoP (6:4:2), Hex: EA: IsoP (6:4:0.3), Hex: EA: IsoP (7:4:0.3), and Hex: EA: IsoP (8:4:0.3).

The circled spots of developed TLC were also observed under UV light (254 nm) (Figure 2). Then, the plate was sprayed with anisaldehyde to allow non-UV compound to be observed. The Rf value for each TLC spot was tabulated in Table 2. The colour of each spot was also recorded after the plate was sprayed with anisaldehyde.

It was found that Hex: EA: IsoP (7:4:0.3) was the most suitable mobile phase for *P. canescens* leaves extracts. Thus, only Rf values of this chromatographic profiles were recorded. The preparative TLC plate of the chloroform extract of *P. canescens* leaves was developed by using Hex: EA: IsoP (7:4:0.3) (Figure 3). The Rf values of the bands from the preparative TLC were recorded (Table 3). Eight (8) bands were observed under UV light ($\lambda = 254$ nm). The numbering of the bands started from the bottom (No. 1) to the upper band (No. 8) of the plate.

4.0 Discussion

Based on Figure 1 and 2, the compound spots from the methanol, chloroform and hexane extracts are present. Thus, all three samples of *P. canescens* leaves extracts contain three types of compounds (i.e. polar, middle polar and non-polar components). In Figure 1, three solvent systems were used as the mobile phase. The three solvents have different polarity to develop different compounds based on their polarity. Hex: EA: $CHCl_3$ (6:4:2) provided less visualized spots than other mobile phases. It was due to the absence of a polar solvent, such as isopropanol. This could explain the retained, concentrated spots on the point of origin on chloroform and methanol extracts. Polar compounds cannot travel to the solvent front if the polar solvent is not present in the mobile phase. Thus, $CHCl_3$ was replaced with isopropanol, and the mixture was modified into Hex: EA: IsoP (6:4:2).

The TLC plate was observed and showed to be too polar due to high volume of isopropanol. All spots were overlapped and did not separate well with each other.

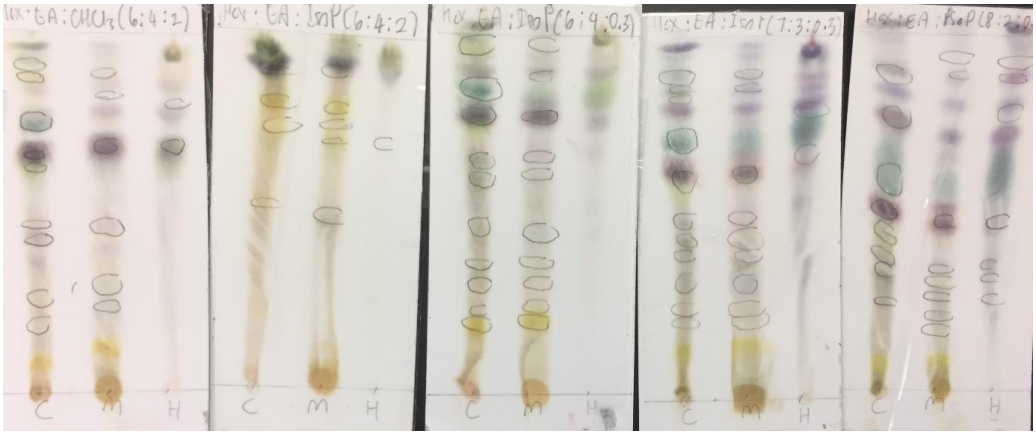
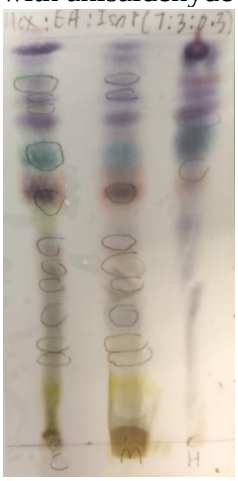


Figure 1. The results of Thin Layer Chromatography. The extracts of *P. canescens* were labelled on the TLC plate as C: Chloroform, M: Methanol, and H: Hexane. The solvents used as mobile phase were labelled on the TLC plate as $CHCl_3$: Chloroform, EA: Ethyl acetate, Hex: Hexane, and IsoP: Isopropanol. Five mobile phases that produced remarkable results; Hex: EA: $CHCl_3$ (6:4:2), Hex: EA: IsoP (6:4:2), Hex: EA: IsoP (6:4:0.3), Hex: EA: IsoP (7:4:0.3), and Hex: EA: IsoP (8:4:0.3). Different mobile phases were able to produce different spots and Rf values. The TLC plate with mobile phase Hex: EA: IsoP (7:4:0.3) was highlighted with red-coloured square box, having the most ideal chromatographic profile.

A) The TLC plate after spraying with anisaldehyde



B) The TLC plate was visualized under UV (254 nm).

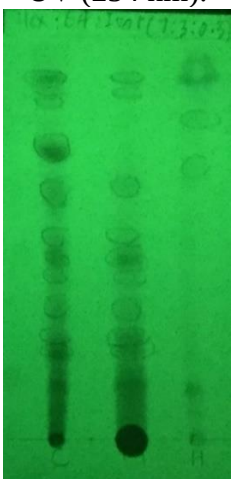


Figure 2. Chromatographic profile of *P. canescens* extract of mobile phase Hex: EA: IsoP (7:4:0.3). The left side of the plate is the chloroform extract. The middle part is the methanol extract, while the right side is the hexane extract.

Table 2. The Rf values of *P. canescens* leaves extracts [Hex: EA: IsoP (7:4:0.3)].

Spots	Rf values					
	Chloroform extract	**Colour**	**Methanol extract**	**Colour**	**Hexane extract**	**Colour**
1	0.23	Yellow	0.23	Yellow	0.68 *	Slight red
2	0.26	Yellow	0.26	Yellow	0.71	Slight purple
3	0.29	Light purple	0.29	Light purple	0.75 *	Purple
4	0.41	Colourless	0.41	Colourless	0.83	Turquoise
5	0.46	Colourless	0.46	Colourless	0.85 *	Purple
6	0.53	Light purple	0.53	Light purple	0.90 *	Purple
7	0.59 *	Light green	0.59	Light green	0.94 *	Red
8	0.64	Dark purple	0.64	Dark purple		
9	0.68 *	Slight red	0.68 *	Slight red		
10	0.75	Turquoise	0.75 *	Turquoise		
11	0.85 *	Purple	0.85 *	Purple		
12	0.88	Light green	0.90	Purple		
13	0.90	Purple	0.94	Slight red		
14	0.94	Slight red				

(*) indicates the non-UV compounds that are visualized by anisaldehyde staining, while others are UV-visualised compounds (wavelength, λ = 254 nm).

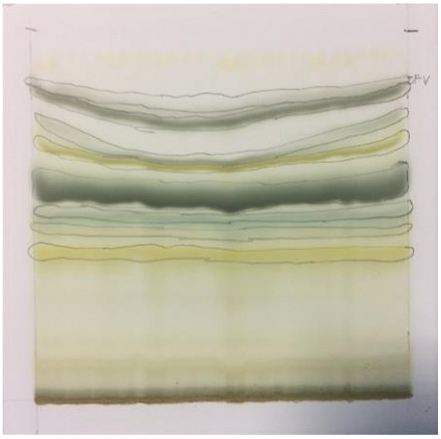


Figure 3. The preparative TLC plate of chloroform extract

Table 3. Rf value of preparative TLC of chloroform extract.

Band	Rf value	Band	Rf value
1	0.39	5	0.68
2	0.47	6	0.76
3	0.53	7	0.82
4	0.59	8	0.88

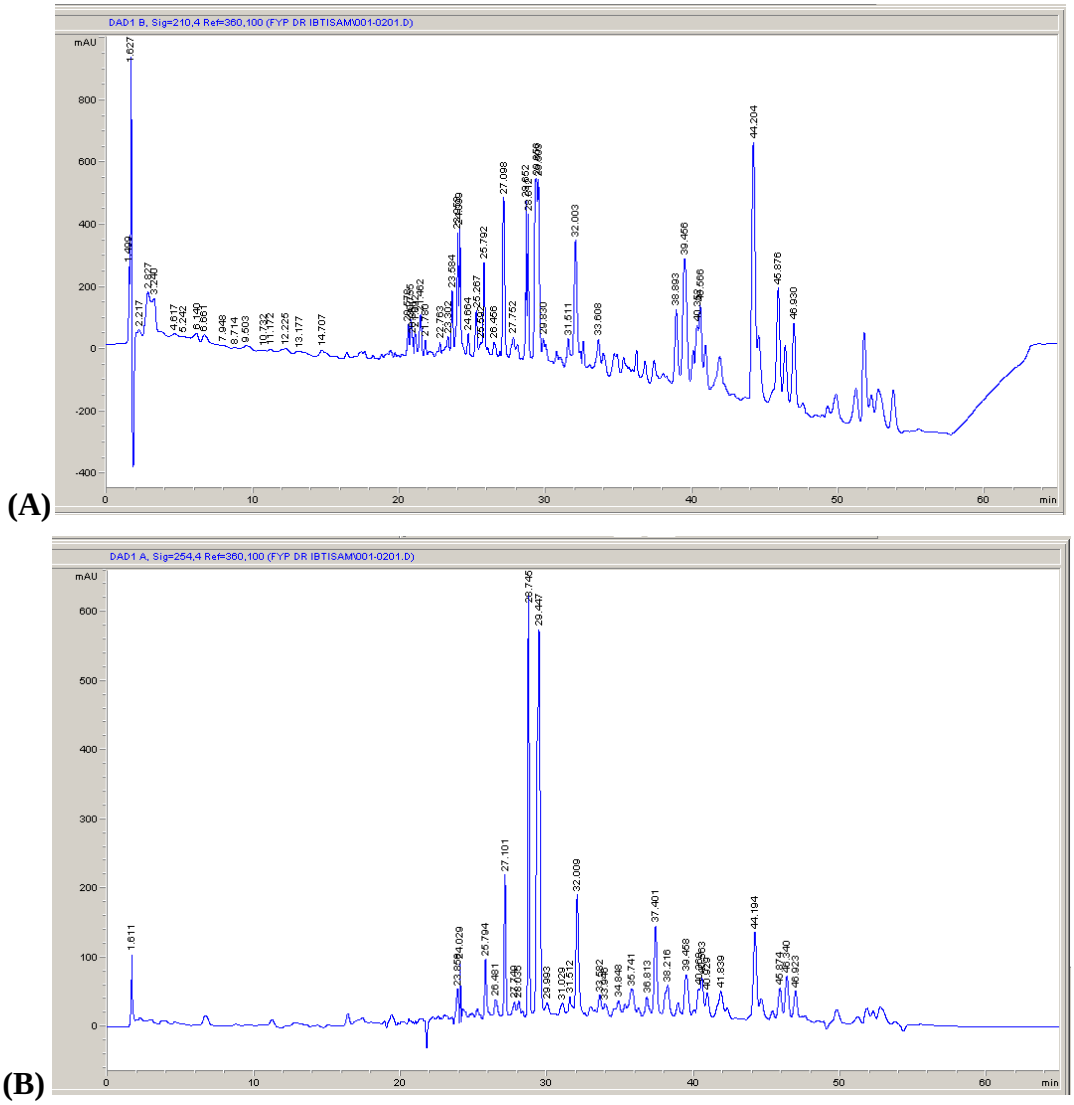


Figure 4. The chromatogram of the methanol extract, λ = 210 (A) and 254 nm (B).

Table 4. The retention time of the peaks from the chromatogram of the methanol extract.

Peak number	(A)Wavelength = 210 nm	(B)Wavelength = 254 nm
	Retention time (min)	Retention time (min)
1	25.792	25.794
2	27.098	27.101
3	28.612	28.745
4	29.859	29.447
5	32.003	32.009
6	38.893	37.401
7	44.204	44.194
8	45.876	45.874

Then, Hex: EA: IsoP with a ratio of (6:4:0.3), (7:3:0.3) and (8:2:0.3) were tested. Hex: EA: IsoP (7:3:0.3) was found to be the better one among those three combinations. It showed better separation of spots and many spots could be developed and visualized. The concentrated spots on the point of origin on chloroform and methanol extracts were also greatly reduced.

In Figure 2, the chloroform extract showed 14 spots indicating the highest number of visualized spots on the plate, as compared to methanol (13 spots) and hexane (7 spots) extracts. In addition, the spots/bands from the chloroform extract showed more intense colour and UV-visible compounds than the methanol extract, at the same Rf value (Table 1). This indicated that *P. canescens* leaves consist of many polar compounds. The chloroform and methanol extracts were more similar to each other, in terms of the phytochemicals in both extracts. Nevertheless, they differ in terms of the quantity of the compounds. The chloroform extract had more compounds that could react with anisaldehyde and showed more intense colour under UV light (λ = 254 nm, Figure 2). This showed that *P. canescens* leaves contain some natural chemical compounds that could dissolve in both polar and non-polar solvent, however, polar solvent is more favourable.

In Figure 2 (B), the chloroform extract showed more spots at different Rf values. This indicating more UV-visible compounds are present, as compared to the methanol extract. Based on Figure 2 (A), the hexane extract has 5 spots that were similar in colour and Rf values, with chloroform and methanol extracts. This may specify that the compounds of that 5 spots were able to be dissolved in three types of solvents. The compounds on the spots possibly belong to different compounds but due to their unique properties, they were able to produce the same colour. Based on Figure 2 (B), the hexane extract is different from other extracts by having two spots at different Rf values, as compared to methanol and chloroform extracts.

The compounds from the chloroform extract of *P. canescens* leaves (Figure 3) were subjected to the ^{1}H-NMR spectroscopy with the hope of finding 7 clerodane-type diterpenoids known as Peronemin A_2, A_3, B_1, B_2, B_3, C_1, and D_1. The ^{1}H-NMR spectroscopic data from Kitagawa *et al*. (1994) was compared with the sample data. Unfortunately, the data did not show any similar peaks or chemical shifts to indicate the presence of peronemins. Thus, re-investigation with higher sample weight is recommended.

The HPLC chromatograms of the methanol extract (Figure 4) were analysed to acquire the number of chemical compounds that could be detected. Based on Figure 4(A), the chromatogram (λ = 210

nm) showed many small peaks that could indicate the presence of some contaminations or very low concentrations of compounds. Meanwhile, the chromatogram 4(B) (λ = 254 nm), showed better baseline separation. Based on Table 4, the HPLC chromatogram of the methanol extract (mobile phase = 0.1% aq. formic acid and acetonitrile, λ = 210 and 254 nm) gave eight major peaks. The peaks at retention time = 39.456 min, 46.960 min and others are less acceptable, due to their low intensity.

5.0 Conclusion

P. canescens leaves were successfully extracted. The best mobile phase for TLC was hexane: ethyl acetate: isopropanol (7:3:0.3). Unfortunately, the NMR data did not signify the presence of peronemins. The HPLC chromatograms showed eight chemical compounds that can be isolated and identified. The purification of the compounds is in progress.

Acknowledgement

Special thanks to the laboratory staff of the Faculty of Pharmacy, UiTM.

Conflicts of interests

The authors declare that there is no conflict of interest regarding the publication of this article.

References

1. Wiart C. Peronema canescens Jack in Medicinal Plants of Asia and the Pacific: CRC Press; 2006. 284-5 p.
2. Ningsih A, Ibrahim A. Aktifitas Antimikroba Ekstrak Fraksi n-Heksan Daun Sungkai (Peronema canescens. JACK) terhadap Beberapa Bakteri dengan Metode KLT-Bioautografi. Journal of Tropical Pharmacy and Chemistry. 2013;2(2):76-82.
3. Matsuura H, Yamasaki M, Yamato O, Maede Y, Katakura K, Suzuki M, et al. Effects of central Kalimantan plant extracts on intraerythrocytic Babesia gibsoni in culture. Journal of Veterinary Medical Science. 2004;66(7):871-4.
4. Kitagawa I, Simanjuntak P, Hori K, Nagami N, Mahmud T, Shibuya H, et al. Indonesian medicinal plants. VII. Seven new clerodane-type diterpenoids, peronemins A2, A3, B1, B2, B3, C1, and D1, from the leaves of Peronema canescens (Verbenaceae). Chemical and pharmaceutical bulletin. 1994;42(5):1050-55.
5. Yani AP, Putranto AM. Examination of The Sungkai's Young Leaf Extract (Peronema canescens) As An Antipiretic, Immunity, Anti plasmodium and Teratogenity In Mice (Mus muculus). International Journal of Science and Engineering. 2014;7(1):30-4.
6. Rosdiana NA, Sari RK. Fraksi Aktif Antioksidan dari Ekstrak Kulit Kayu Sungkai (Peronema canescens Jack.). Bogor Agricultural University: Bogor Agricultural University; 2014. 1-25p.
7. Widodo H, Sismindari S, Asmara W, Rohman A. Antioxidant activity, total phenolic and flavonoid contents of selected medicinal plants used for liver diseases and its classification with chemometrics. Journal of Applied Pharmaceutical Science. 2019;9(06):099-105.
8. Md Pisar M. Medicinal Forest Species in FRIM. Md Pisar M, Mat Ali R, Mohamad Ali NA, editors: Publications Branch, FRIM; 2018. 99-106p.