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Article

Isolation of Bioactive Compound in Methanolic Extract of Putat Leaves (*Planchonia valida*) as Antioxidant

Faizar Farid[1], Vera Lorensya Br Sembiring[1], Nelson[1], Muhammad Furqon Novryan Saputra, Silvi Ayudiah Putri[1], Indra Lasmana Tarigan[1,2], Madyawati Latief[1,2*]

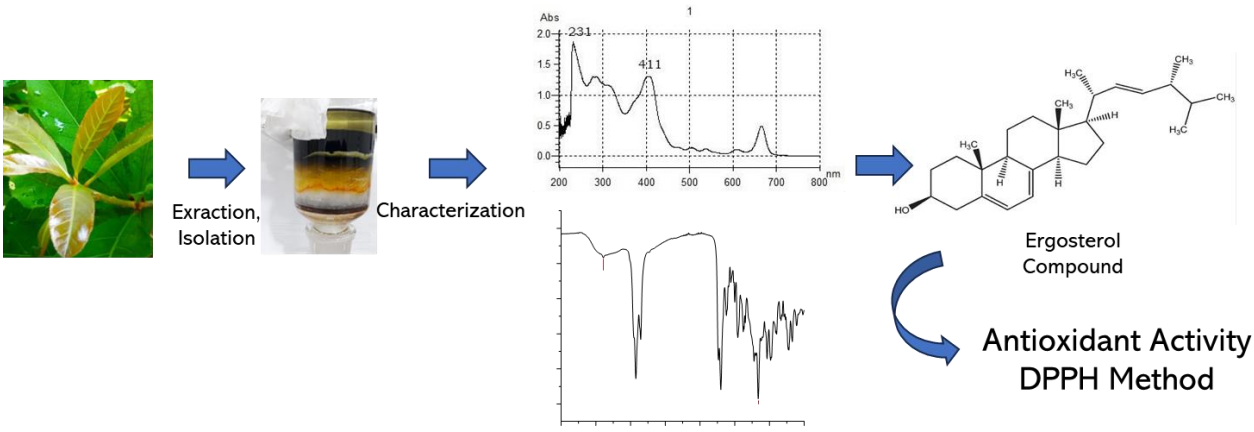
[1]Department of Chemistry, Faculty of Science and Technology, Universitas Jambi, Indonesia
[2]Natural Product and Bioactive Compounds Laboratory, Faculty of Science and Technology, Universitas Jambi, Indonesia

Abstract

Putat leaves (Planchonia valida) are a plant that is traditionally used by people in the Tanjung Lanjut areas, Jambi as fresh vegetables, and as a traditional medicine to treat skin diseases and restore health. This identifies that the putat plant contains chemical compounds that have the potential to have antioxidant activity. Extraction was carried out using n-hexane and ethyl acetate solvents using multistage maceration. Next, phytochemical screening and antioxidant activity tests are carried out to obtain information about fractions that can potentially proceed to the isolation stage. In the maceration process, an extract percentage was obtained with a yield value of 1.39%. Next, isolation was carried out using vacuum liquid chromatography. The isolates obtained were then characterized using UV-Vis and FT-IR spectrophotometer instruments. The isolate obtained was in the form of a wet solid, and if seen through characterization, it belonged to the class of steroid compounds; it was suspected to be an ergosterol compound. Antioxidants are known to have the ability to inhibit and reduce the presence of free radicals in the human body. In this study, antioxidant activity was tested using the DPPH (2,2-diphenyl-1-picrihidazil) method on F1 isolates. The F1 isolate obtained was proven to have antioxidant activity with a value of 99.2265 ppm, which is classified in the strong antioxidant activity range.

Keywords: *Antioxidant Activity, Isolation, and Putat Leaves*

Graphical Abstract



* Corresponding author
Email addresses: madyawatilatief@unja.ac.id

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Introduction

Free radicals are molecules that have unstable and reactive properties, due to they have one or more unpaired electrons. Free radicals can attack vulnerable compounds such as lipids and proteins, which will eventually cause dangerous diseases [1]. The free radical material is a compound or molecule that contains one or more unpaired electrons in its outer orbital. The existence of an unpaired electron makes the compound very reactive to find its partner. The trick is to bind or attack the electrons of the molecules around it. What free radicals bind to are generally large molecules such as lipids, proteins, and DNA (carriers of traits). If this happens, it will cause cell damage that cannot be controlled [2]. Antioxidants can neutralize free radicals through electron donors so that free radicals are more stable and unreactive [3]. Therefore, antioxidants are needed in order to protect the body from free radicals and reduce their negative impact on the body [4].

Based on observations in Muaro Jambi district, precisely in the village of Tanjung lanjut, Putat (*Planchonia valida)* plants grow in many waters. This plant is not widely used in the community, only used as tea. Putat is a hardwoodcommonly used to build buildings, house floors, wall panels, and tool holders. The wood is also suitable for use as firewood. Putat leaves and young shoots are usually eaten, used for vegetables by steaming [5].

The plant part used from the putat plant is the leaves. Putat leaves can be used to treat skin diseases such as itching by pounding the leaves and then attaching them to the itchy area. The group of secondary metabolite compounds contained in the ethanol extract of putat leaves are Alkaloids, Flavonoids, Tannins, Saponins and Steroids [6].

Based on previous research through phytochemical screening of putat leaf powder simplisia *(Planchonia valida)* states that it positively contains all chemical compounds, namely alkaloids, tannins, flavonoids, steroids, and saponins [7]. This statement is also supported by research conducted by Supriningrum *et al* [6] which explains that alkaloids, flavonoids, tannins, saponins and steroids are among the chemical components contained in putat leaves. Research from the *Planchonia* genus has been reported including phytochemical tests of saponin compounds acylated with triterpenoids from *Planchonia careya* species [8]. It has been isolated 6 chemical compounds from *Planchonia careya* that have antibacterial activity against (+)-galocatechin, galocatechin-(4α→8) galocatechin, α-dimorphenolic acid, hiptata acid, 3-β-Otrans-p-cumaroiltormentic acid, 3-β-O-cis-p-cumaroiltormentic acid. Ethanol extracts are known to be positive based on phytochemicals are alkaloids, phenolics, saponins and flavonoids. Screening of secondary metabolite compound profile of the extract found major components such as Eudesolbovatol A, Hypercalin B, Propofol Glucuronide, 2,3-Dinor-6, 15-diketo-13, 14-dyhydro-20-carboxyl-PGF1a. From the results of existing research that putat leaves contain flavonoid compounds, flavonoid compounds are secondary metabolite compounds of the polyphenol group that have the ability to act as antioxidants by counteracting free radical compounds. Therefore, new natural antioxidant compounds must continue to be sought or at least renewed in order to be a safer antidote for free radicals. The search for natural antioxidant compounds is directed towards natural resources [9].

Material and Methods

Materials and Equipments

The materials used are Putat leaves *(Planchonia valida)* obtained from one of the villages in Muaro Jambi district, namely Tanjung Lanjut Village, Methanol, Ethyl Acetate, Aquadest (Sigma Aldrich), N-Hexane, Silica Gel 40, Silica Gel 60 TLC Plate, $HgCl_2$, KI, Mayer Reagent, Dragendrof Reagent, Bismuth 3 Nitrate, Concentrated Nitric Acid, Bouchardat Reagent, I_2, HCl, Magnesium Powder, Amyl Alcohol, Iron (III) Chloride Reagent, DPPH Powder, Standard Solution, Vitamin C (ascorbic acid).

The equipments and instrumentations used in this study were maceration bottles, filter paper,

rotary evaporator components, funnels, measuring cups, Erlenmeyer cups, VLC and GCC columns, vacuum pumps, capillary tubes, TLC plates (Merck), drip plates, test tubes, test tube rack, dropping pipette, 1 ml micropipette, 10 ml micropipette, KBR pellet, volumetric flask, vial, stir bar, UV-Vis spectrophotometer (Shimadzu, Japan), FTIR spectrophotometer (Bruker, USA)

Methods

Sample Preparation

The sample to be studied is the leaf part of the putat plant (*Planchonia valida*). The leaves are cleaned of dirt and mud and then aerated indoors for 7 days to reduce the water content contained in the putat leaves. Then the dried samples are finely ground or blended until smooth and then the powdered putat leaves that have been pureed are stored in a clean container, protected from sunlight and at room temperature to avoid contamination from air and light. A dry sample of 1.4 kg was macerated in stages. The advantage of extraction using the maceration method is that it can be carried out at room temperature, thus reducing the possibility of compounds in the extract to degrade. Meanwhile, multistage maceration is carried out to separate compounds in the sample according to their level of polarity. The initial solvent used for maceration was n-hexane, as much as 1.5 L. Then, maceration was continued with ethyl acetate solvent. Ethyl acetate extract. Then, continued with methanol solvent. Maceration was carried out for 2 x 24 hours and the obtained meserat was concentrated with a *rotary evaporator* until a thick extract was obtained.

Phytochemical Screening

Alkaloids; This was done mayer reagent methods and dragendrof reagent. *Flavonoids;* samples to be tested Addition of Mg powder and concentrated HCl positive results when formed color changes to dark red or orange. *Flavonoids***:** samples to be tested Addition of Mg powder and concentrated HCl positive results when formed color changes to dark red or orange. *Tannins***:** give positive results, namely the formation of a blackish green color after the addition of FeCl reagent$_3$ 1%. *Saponins***:** give positive results, namely the formation of permanent foam after shaking and with the addition of 2N HCl the foam does not disappear. *Terpenoids or steroids***:** in plants are tested using Liebermann-Burchard reagent (anhydrous acetic acid and concentrated sulfuric acid) which will give an orange red or purple color for terpenoids and bluish green for steroids [10].

Compound Separation and Purification

Column Chromatography: Liquid vacuum column chromatography (KVC) was performed using silica gel as stationary phase with silica gel sample ratio (1:20). The results of column chromatography were TLC again. Rf values on the chromatogram are combined if they have identical stains

Thin Layer Chromatography (TLC): All fractions were subjected to TLC test. Fractions with the same spot stain were pooled and analyzed with a solvent ratio of N-hexane: Methanol (7:3). Vials that had the same stain were combined.

Characterization of Isolated Compounds

UV-Vis spectrophotometer. A total of 2 ml of isolate was put in a cuvette and observed the spectrum at a wavelength of 200-800 nm to identify the absorbance value of active compounds at the maximum wavelength [13]

FTIR spectrophotometer. 0.2 g of KBr pellet was added with 1 drop of isolate, dried then identified with FTIR spectrophotometer at wave numbers 4000-400 cm^{-1}.

Determination of Extract Yield Percentage

Determination of the percentage yield of the extract was calculated using equation 1.

$$\% \text{ Yield} = \frac{\text{Extract Weight (gr)}}{\text{Weight Of Raw Materials (gr)}} \text{ x } 100\% \qquad (1)$$

Determination of Phytochemical Analysis

Analysis of phytochemical content is done by looking at changes in the reaction that occurs between the sample tested and the reagents used. Changes in the reaction in question

include color changes, precipitates formed and the presence of layers formed.

Determination of Antioxidant Activity

% inhibition value. The % inhibition value was determined using equation 2.

$$\%\ inhibition = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100\% \quad (2)$$

IC value$_{50}$. To calculate the IC value$_{50}$ can be obtained by plotting a graph of the relationship between sample concentration and % inhibition. The x-axis is the sample concentration while the y-axis is the % inhibition. Then calculate the regression equation y = a + bx where to calculate the IC value$_{50}$ can be known from replacing the y value in the regression equation with a value of 50 (Equation 3).

$IC_{50} = (50 - a)/b$ (3)

y = % inhibition (50)

a = Intercept (Line intersection on the y-axis)

b = Slope

x = Concentration

Result And Discussion

Extraction

From the results of n-hexane maceration, a greenish yellow extract was obtained. Then, maceration was continued with ethyl acetate solvent. The ethyl acetate extract obtained is green in color. Then, continued with methanol solvent, from the results of the maceration obtained the results obtained a concentrated green extract. The extract obtained from the methanol solvent was then concentrated using a rotary evaporator. From the methanol extract, a dry extract of 19.5 g was obtained, with a yield of 1.39%. The dried methanol extract has a sticky character like resin and is blackish green in color. Phytochemical screening was carried out with the aim of knowing preliminary information about the profile or content of phytochemical compounds contained in the methanol extract of putat leaves. The results of phytochemical screening of putat leaf methanol extract can be seen in Table 1. It is known that methanol extract of putat leaves contains active compounds of flavonoids, tannins and steroids.

Table 1. Phytochemical screening results of methanol extract of putat leaves

Secondary Metabolites	Results
Alkaloids	-
Flavonoids	+
Saponins	-
Tannin	+
Steroids	+

Antioxidant Activity of *Planchonia valida*

The results of the antioxidant activity test of putat (*planchonia valida*) leaf extract can be seen in Table 2 below:

Table 2: Antioxidant Activity

Samples	IC_{50} (ppm)	Description
Methanol Extract	15.71	Very Strong
Ascorbic Acids	14.9	Very Strong

From the results of quantitative testing on the methanol extract of putat leaves *(Planchonia valida)* using the DPPH method, the IC_{50} value was obtained at 15.745 ppm. Indication of the intensity of antioxidant power is very strong and has potential as an antioxidant [13]. a compound is said to be a very strong antioxidant if it has an IC_{50} value < l0 µg/mL, a strong antioxidant if the I_{C50} value is between 10-50 µg/mL, moderate if the IC_{50} value ranges between 50-100 µg/mL, weak if the IC_{50} value is between 100-250 µg/mL and inactive if the IC_{50} is above 250 µg/mL [11].

Isolate Investigation

From the isolation process carried out, 22 vials were obtained based on the color of the resulting band. Furthermore, thin layer chromatography (TLC) tests were carried out by observing the same stain pattern from each vial tested to identify the combined fraction. It can be seen in Table 3 the results of grouping fractions of kvc results of methanol extract of putat leaves *(Planchonia valida).*

Based on the grouping, a qualitative test of antioxidant activity was carried out again on the fractions obtained by re-bottling each fraction on TLCTLC and eluting using the mobile phase n-hexane: ethyl acetate (Figure 1).

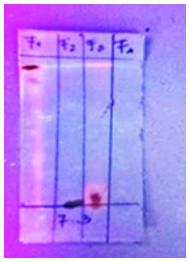


Figure 1. Qualitative antioxidant activity test of KVC fraction using DPPH spray on methanol fraction.

After spraying DPPH on the four fractions, the results showed that there was a yellow background on F1 which was more dominant so that the fraction that was continued was the F1 fraction. Furthermore, purification is carried out by recrystallization using solvents that are nonpolar (n-hexane), semi-polar (ethyl acetate), polar (methanol). After that dried and obtained isolates (Figure 2).

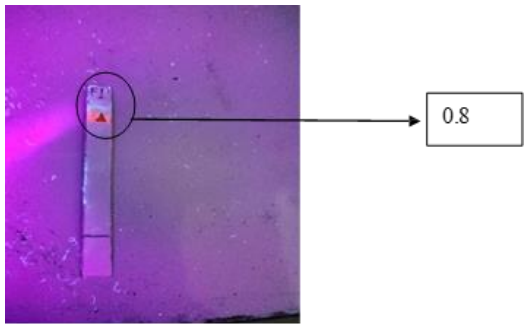


Figure 2. TLC results of Crystal F1 (methanol KVC results) using methanol eluent: N-hexane.

Table 3. Grouping of Fractions

Fractions	Vial Number	Weight (mg)
1	3	85
2	4-7	76
3	8-13	55
4	14-22	68

Characterization of Isolated Compounds

Characterization through Phytochemical Screening. Phytochemical screening is carried out to find out preliminary information about the description of the compound content contained in the F1 isolate of putat leaves *(Planchonia valida).* Can be seen in Table 4 F1 methanol isolate phytochemical screening results.

Table 4. Phytochemical screening results of F1 methanol isolate

Phytochemical screening	Test reagent	Description	Results
Alkaloids	Dragendrof	Orange precipitate	-
Flavonoids	Mg + HCl	Red/orange precipitate	-
Saponins	Hot water + HCl	Forms a stable foam	-
Tannin	$FeCl_3$	Formed blackish green color	-
Steroids	Lieberman burchard	Formed green/blue color	+

The working principle of the UV-Vis spectrophotometer is the interaction between radiation at a wavelength of 200-800 nm which is passed to a compound. This interaction produces a transition between electronic energy in organic molecules. The purpose of this characterization by UV-Vis spectrophotometer aims to analyze compounds that contain chromophore groups, which are part of molecules that absorb UV and visible light. In the figure shows the maximum absorption at the wavelength, the UV-Vis spectrum shows that the isolate gives two absorption peaks, namely in band 1 λ = 231 nm and band 2 λ = 411 nm. The absorption at 231 indicates the presence of a conjugated diene/rangacap system in the

structure. While at 411 nm the possibility of an electronic transition of a C = C chromophore.

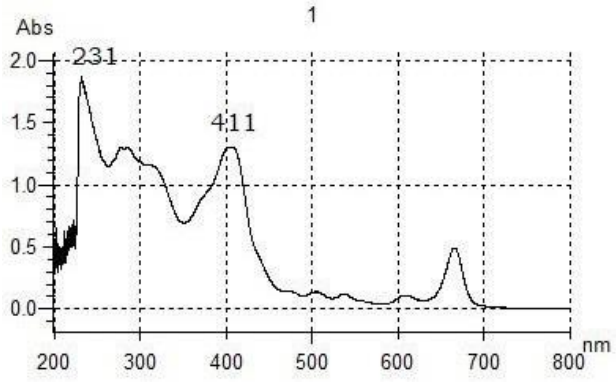
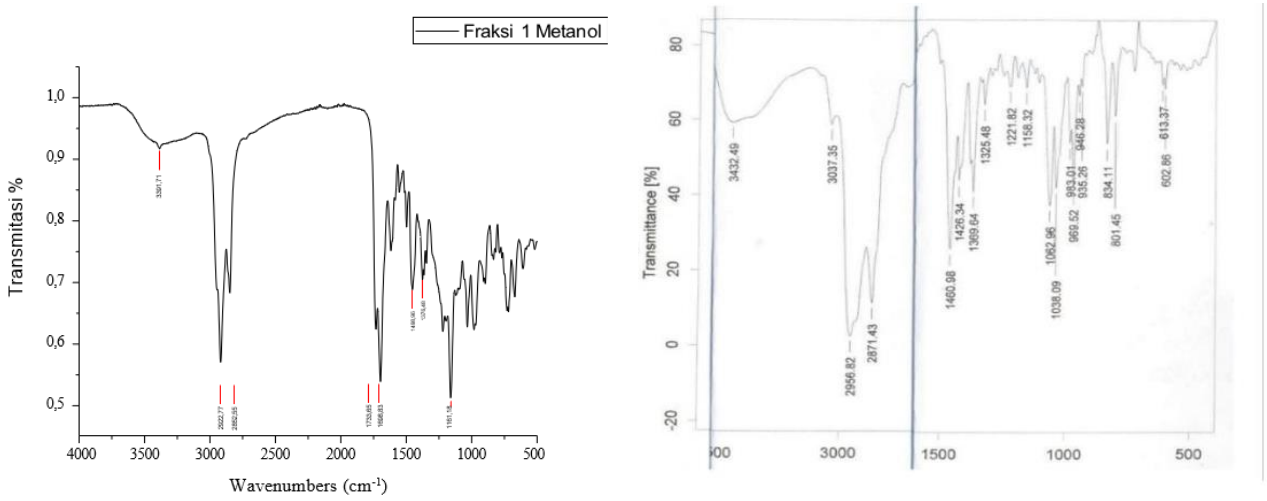


Figure 3. UV-Vis spectrum of F1 methanol isolate

IR Spectrum of Isolate

Characterization using an IR spectrophotometer in this study was carried out to determine the presence of functional groups in the isolate obtained. Can be seen in Figure 4 FTIR spectrum of F1 methanol isolate [14].

Identification of the isolate by IR spectrophotometry showed the presence of absorption at wavenumber 3391.71 allegedly an absorption of the O-H group. this conjecture is strengthened by the presence of absorption in the ν (max) 1161.18 cm^{-1} region which indicates C-OH absorption. There are also vibrations at ν (max) 2922.16 cm^{-1} and 2852.55 cm^{-1} allegedly an absorption of the C-H group. This conjecture is reinforced by the bending vibrations at ν (max) 1498.96 cm^{-1} which indicates the presence of CH groups



(a) **(b)**

Figure 4. FTIR spectrum of F1 methanol isolate (a) Pure ergosterol [14] (b)

The absorption band at ν (max) 1376.94 cm^{-1} indicates the bending of the C-H group of CH_3. The absorption band at ν (max) 1698.83 indicates the presence of non-aromatic C = C (ena) groups. The absorption band at ν (max) 1733.65 cm^{-1} is thought to be an absorption for the C=O group, as reported by Hiroshi *et al*. (1976) there is an O-H (hydroxyl) bond in ring position A and has a C=C (ena) double bond and a C=O (carbonyl) bond in ring position B in the steroid compound makisteron A and makisteron D in *diplazium donianum*. The presence of O-H (hydroxyl) bonds, having a C=C (ena) double bond and a C-O (carbonyl) double bond indicates that isolate F1 is a steroid compound.

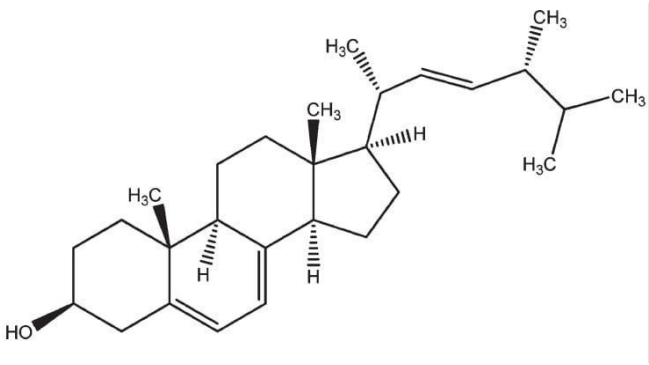


Fig 5. Ergosterol structure [16].

Steroid compounds have similar FT-IR spectrum patterns with the compound is ergosterol from the literature can be seen that 11 b and c. Functional groups contained in the FT-IR spectrum of the isolate in accordance with the characteristic functional groups that exist in ergosterol compounds [17-18].

Antioxidant Activity of Isolated Compound

After obtaining pure isolate, antioxidant activity test was conducted quantitatively on the sample to determine the value of antioxidant activity so that it can be determined the range of antioxidant strength levels in F1 isolate. Antioxidant activity test was conducted using DPPH method using varied sample concentrations starting from 10 ppm, 30 ppm, and 50 ppm [18]. In addition, testing was also carried out on ascorbic acid which acts as a positive control. This aims to compare the strength of antioxidant activity in the sample with commercial pure compounds that have been commonly used as antioxidant agents. It can be seen in Table 5 the results of F1 antioxidant activity test.

Table 5. Antioxidant activity of F1 Isolate

Samples	IC_{50} (ppm)	Activity
Isolate	99.22	Strong
Ascorbic Acids	14.9	Very Strong

To quantitatively determine the antioxidant activity of a sample, a linear regression equation is required to determine the IC_{50} . $_{50}$From the results of the antioxidant activity test in Table 5 conducted on isolate F1 and ascorbic acid, it is known that isolate F1 has an IC value of 99.2265 ppm. This indicates that isolate F1 has a strong antioxidant level [20]. This is reinforced by comparing the IC $value_{50}$ isolate with IC_{50} ascorbic acid, which is 14.9488 ppm. Where the smaller the IC_{50} value of a sample, it means that the level of antioxidant activity is stronger.

Conclusion

Methanol extract of *Planchonia valida* contains steroid compounds, flavonoids and tannins. Antioxidant activity of putat leaf extract (*Planchonia valida)* and methanol fraction 1 of putat leaf (*Planchonia valida) is* expressed in IC $value_{50}$ which is categorized in strong and strong antioxidants. The results of the characterization of isolates predicted from the 1 methanol fraction of putat leaves (*Planchonia valida)* are ergosterol compounds.

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Author Contributions

Conceptualization, FF and ML.; Methodology, VLS and ILT; Software, MFNS and SAP.; Validation: FF and VLS; Formal Analysis, ML and ILT.; Investigation, VLS and ILT; Resources, FF and N.; Data Curation, FF and N.; Writing – Original Draft Preparation, VLS, MFVS; Writing – Review & Editing, ILT, ML; Visualization: SAP, ML.;

Supervision, FF and ML; Project Administration, ML.

Conflic of Interest

The authors declare no conflict of interest

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