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Research Article

GC-MS analysis of bioactive compounds and antibacterial activity of nangka leaves (*Artocarpus heterophyllus* Lam)

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Abstract

Nangka is a plant that has various kinds of potential both because of the nutritional content provided through the fruit and also part of the content of bioactive compounds contained in the leaves. This study aims to identify the content of bioactive compounds and determine their content using GC-MS and determine the potential antibacterial activity against *E.coli*, *S. aureus*, *S. epidermidis*, *S. typhi*, *P.acnes* from the ethanol extract of nangka leaves (*Artocarpus heterophyllus* Lam). Screening results showed positive containing phenolic groups, flavonoids, tannins, saponins, and alkaloids. The results of determining the content of bioactive compounds for phenolics, tannins and flavonoids were 27.654±0.054 mg GAE/g d.w ethanolic extract, 0.46±0.017 mg TAE/g d.w ethanolic extract and 2.978±0.192 mg QE/g d.w ethanolic extract. GC-MS analysis showed the content of octadecanoic acid with a retention time of 36.489 minutes with a concentration of 29.91% and the ethanolic extract of nangka leaves had good potential activity as an antibacterial.

Keywords

Artocarpus heterophyllus, Tannins, Phenolics, Flavonoids, Antibacterial, GC-MS

Introduction

Nangka is the local name used for *Artocarpus heterophyllus*, Lam (latin name) in the Batak tribe of North Sumatra Province, Indonesia. This plant belongs to the Moracea family which is commonly found in subtropical and tropical countries, especially in Southeast Asia (Liu et al. 2020; Khan et al. 2021). Nangka plants have only been used as plants that produce fruit for consumption, because of the high nutritional content contained in the fruit and good for health (Fu et al. 2020). Besides the fruit, the leaves of this plant are also reported to have various pharmacological benefits, including in the treatment of asthma, diarrhea, anemia and dermatitis (Vázquez-González et al. 2020), antioxidant, antifungal, antitumor, anticancer, antidiabetic, anti-inflammatory, tyrosinase inhibitor, antibacterial, antiproliferative (Liu et al. 2020; Sreeja Devi et al. 2021) and antimalarial (Shukla and Kashaw 2019). The pharmacological potential given is supported by the content of secondary metabolite compounds, including phenolic acids, flavonoids, terpenoids, stilbenoids (Gurning et al. 2020; Pranay et al. 2021).

The content of the secondary metabolite compound that is most widely reported to have the potential to be used in various treatments is the flavonoid, tannin, and phenolic groups. For example, the phenolic and flavonoid

groups are widely reported to be responsible for various degenerative diseases caused by free radicals (Tungmunnithum et al. 2018; Cosme et al. 2020; Jucá et al. 2020; Mutha et al. 2021), and tannins in various treatments caused by microbes (Girard and Bee 2020; Molino et al. 2020; Fraga-Corral et al. 2021). Based on this information, the group of phenolic compounds, flavonoids, tannins and the analysis of the class of compounds contained in the leaves extracted with ethanol were carried out and the activity was determined as antibacterial.

Materials and methods

Materials

The tools used were laboratory glassware, Whatman filter paper No. 1, hotplate, rotary evaporator (Heidolph), petri dish, wire loop, aluminum foil, tweezers, spray bottle, oven (Memmert UN55), laminary air flow, vortex (Cole Parmer), spreader/L glass, incubator (Memmert IN55), spectrophotometry (Genesys 10S-UV VIS), GC-MS (Shimadzu, QP2010S), SPPS version 27, ingredients including Chloramphenicol, blank disc paper (Oxoid), Mueller Hinton Agar (MHA) (Oxoid CM0337), Dimethyl Sulfoxide (DMSO 10%) (Merck), Mc Farland 0.5%, standard reagents for phytochemical screening, *Escherichia coli (E. coli)*, *Staphylococcus aureus (S. aureus)*, *Salmonella typhi (S. typhi)*, *Staphylococcus epidermidis (S. epidermidis)*, and *Propionibacterium acnes (P. acnes)*.

Preparation of ethanol extract of nangka leaves (*Artocarpus heterophyllus* Lam)

The leaves samples used were fresh, dark green, and good from nangka trees that were already fruiting. The leaves were washed and cleaned in running water, drained, and dried in a sample drying cabinet at 60 °C. The dried leaves samples were powdered with a magnetic blender. The powder was extracted using ethanol solvent by maceration method for 3 days, then filtered using whatman filter paper no 1. The residue was extracted again with a new solvent for 2 days and then filtered. Each macerate was concentrated using a vacuum rotary evaporator at 60 °C and combined. The thick macerate was placed in a glass bottle and stored in the sample cabinet in the pharmacognosy laboratory before the next step.

Phytochemical screening

Phytochemical screening was carried out to qualitatively determine the class of secondary metabolite compounds contained in the sample. Phytochemical screening includes flavonoid, phenolic, tannin, alkaloid, saponin, steroid and triterpenoid compounds using standard reagents (Gurning 2020; Gurning et al. 2020; Silaban et al. 2022).

Determination of the total group of secondary metabolites

Phenolic content

Determination of the class of total phenolic compounds contained in the ethanolic extract of nangka leaves by colorimetric method using Folin-Ciocalteou which was measured using UV-Vis spectrometry at a maximum wavelength of 765 nm (gallic acid wavelength as standard). 250 µL of nangka ethanol extract from 1000 ppm mother liquor was mixed with 400 µL of Folin-Ciocalteou in a 10 mL volumetric flask, allowed to stand for 5 minutes. Once achieved, the addition of a solution of 4 mL of 10% Na_2CO_3 and distilled water until the mark is 10 mL. The solution mixture was homogenized by centrifugation, then allowed to stand for 30 minutes, then the absorbance was measured by UV-Vis spectrometry (Šafranko et al. 2021; Sousa et al. 2021; Sinaga et al. 2022). Total phenolic was determined equivalence with gallic acid as standard. The standard curve for gallic acid is determined by the concentration (50–150 ppm). Standard line linear regression equation ($y = 0.0037\times + 0.0056$; $R^2 = 0.9501$), where y= absorbance and x = gallic acid concentration. The total phenolic content was expressed in mg gallic acid equivalent/gram dry weight ethanolic extract. All measurements were carried out in three times repetition.

Flavonoids content

Determination of the class of total flavonoid compounds contained in the ethanolic extract of nangka leaves by colorimetric method using 10% AlC_3 which was measured using UV-Vis spectrometry at a maximum wavelength of 431 nm (quercetin wavelength as standard). 250 µL of nangka ethanol extract from 1000 ppm mother liquor was mixed with 200 µL of 10% AlC_3 and distilled water to the mark of a 10 mL volumetric flask. The solution mixture was homogenized by centrifugation and allowed to stand for 30 minutes at room temperature, then the absorbance was measured by UV-Vis spectrometry (Šafranko et al. 2021; Sousa et al. 2021; Sinaga et al. 2022). Total flavonoids were determined equivalence with quercetin as standard. The standard curve of quercetin is determined by the concentration (25–200 ppm). Standard line linear regression equation ($y= 0.0006\times + 0.0024$; $R^2= 0.9977$), where y = absorbance and x = quercetin concentration. The total flavonoid content was expressed in mg quercetin equivalent/gram dry weight ethanolic extract. All measurements were carried out in three times repetition.

Tannin content

Determination of the class of total tannin compounds contained in the ethanolic extract of nangka leaves by colorimetric method using Folin-Ciocalteou which was measured using UV-Vis spectrometry at a maximum wavelength of 760 nm (tannic acid wavelength as

standard). 200 µL of nangka ethanol extract from 1000 ppm mother liquor was mixed with 200 µL of Folin-Ciocalteou in a 5 mL volumetric flask, allowed to stand for 5 minutes. Once achieved, the addition of a solution of 100 µL of saturated Na_2CO_3 and distilled water was continued to the mark of 5 mL. The solution mixture was homogenized by centrifugation, then allowed to stand for 40 minutes, then the absorbance was measured by UV-Vis spectrometry (Gurning et al. 2021; Patra et al. 2021; Salih et al. 2021; Tandi et al. 2021). Total tannin was determined equivalence with tannic acid as standard. The standard curve for tannic acid is determined by the concentration (100–600 ppm). Standard line linear regression equation (y= 0.0694× +0.0334; R^2= 0.9989), where y = absorbance and x = concentration of tannic acid. The total tannin content is expressed in mg tannic acid equivalent/gram dry ethanolic extract. All measurements were carried out in three times repetition.

Component analysis with GC-MS

Analysis of secondary metabolites contained in the ethanol extract of nangka leaves (*Artocarpus heterophyllus* Lam) by GC-MS using GC-MS-QP2010S Shimadzu type equipment with column type DB-5MS with dimensions of 30 meters × 0.25 mn ID × 0.25µm. The carrier gas used was helium with an ionization of 70 Ev, injector temperature of 300 °C, flow rate of 0.52 mL/min, programmed column temperature of 70 °C which was then slowly increased by programmed increments of 5 °C until the temperature was maintained at 300 °C. Identification of compounds is based on similarities to the Willey compound libraries (Gurning et al. 2020; Ojha et al. 2022; Paudel et al. 2022).

Determination of activity as antibacterial

Antibacterial activity was determined by observing the diameter of the clear zone around the disc paper which had been induced by various concentrations of ethanolic extract of nangka leaves (*Artocarpus heterophyllus* Lam) according to the scratch plate method. The media used in culturing the bacteria *Erscherichia coli (E. coli)*, *Staphylococcus aureus (S. aureus)*, *Salmonella typhi (S. typhi)*, *Propionibacterium acnes (P. acnes)* and *Staphylococcus epidermidis (S. epidermidis)* Muller Hinton Agar (MHA)) are sterile. The solvent used in making each extract concentration was DMSO. DMSO 10% negative control and chloramphenicol as positive control. A solution of 20 mL of MHA media was put into each petri dish, then allowed to solidify at room temperature. The suspension of each bacterium was taken one loop and scratched in a circle on the media evenly using a cotton bud. Then each concentration of ethanol extract of nangka leaves that had been incubated on disc paper was put into a petri dish and allowed to stand at room temperature (37°C) for 24 hours. Then the antibacterial activity was determined from the clear zone (inhibition zone) which was measured with a caliper. Repeats and measurements were three times repetition (Ghosh et al. 2008; Kasta 2020; Harahap et al. 2021). The significance level between treatments was determined using a one-way ANOVA analysis with $p < 0.05$.

Results and discussion

The thick ethanol extract of nangka leaves (*Artocarpus heterophyllus* Lam) was obtained from maceration at room temperature which was concentrated at 60 °C using a heidolp brand rotary vacuum evaporator with a speed of 90 rpm. The results of phytochemical screening of various groups of bioactive compounds are presented in Table 1.

Table 1. Phytochemical group screening of nangka leaves ethanol extract.

No.	Group of compounds	Standard reagents	Occurrence
1.	Phenolics	$FeCl_3$ 5% $_{\text{at ethanol}}$	Present
2.	Flavonoids	Shinoda test (Mg + HCl $_{\text{(concentrated)}}$	Present
3.	Tannins	$FeCl_3$ 5%	Present
4.	Alkaloids	Dragendorff	Present
5.	Steroids/ triterpenoids	Liebermann Bouchard	Present
6.	Saponins	Foaming test	Present

The content of the identified group of phytochemical compounds supports the ability for various diverse pharmacological effects where the flavonoid content is reported to have the ability as a free radical inhibitor (antioxidant) and potential as an antidiabetic, saponins as cholesterol lowering and lowering glucose in the blood, tannins have the function of accelerating wound healing, alkaloids. thought to synergize with tannins and phenolics as antidiabetic (Ajiboye et al. 2018). The phenolic content obtained quantitatively from the ethanolic extract of nangka leaves was 27.654±0.054 mg GAE/g d.w ethanolic extract measured at a wavelength of gallic acid (765 nm) with the standard solution. The tannin content obtained was 0.46 ± 0.017 mg TAE/g d.w ethanolic extract which was measured at the maximum wavelength of tannic acid solution (745 nm) as the standard solution. The flavonoid content was 2.978±0.192 mg QE/g d.w ethanolic extract which was measured at the maximum wavelength of quercetin solution (431 nm) as the standard solution.

The ethanol extract of nangka leaves (*Artocarpus heterophyllus* Lam) showed potential antibacterial activity which was observed from the formation of a measurable clear zone. The antibacterial activity ability of the measured clear zone for various specified concentrations showed strong criteria as antibacterial (Table 2, Fig. 1).

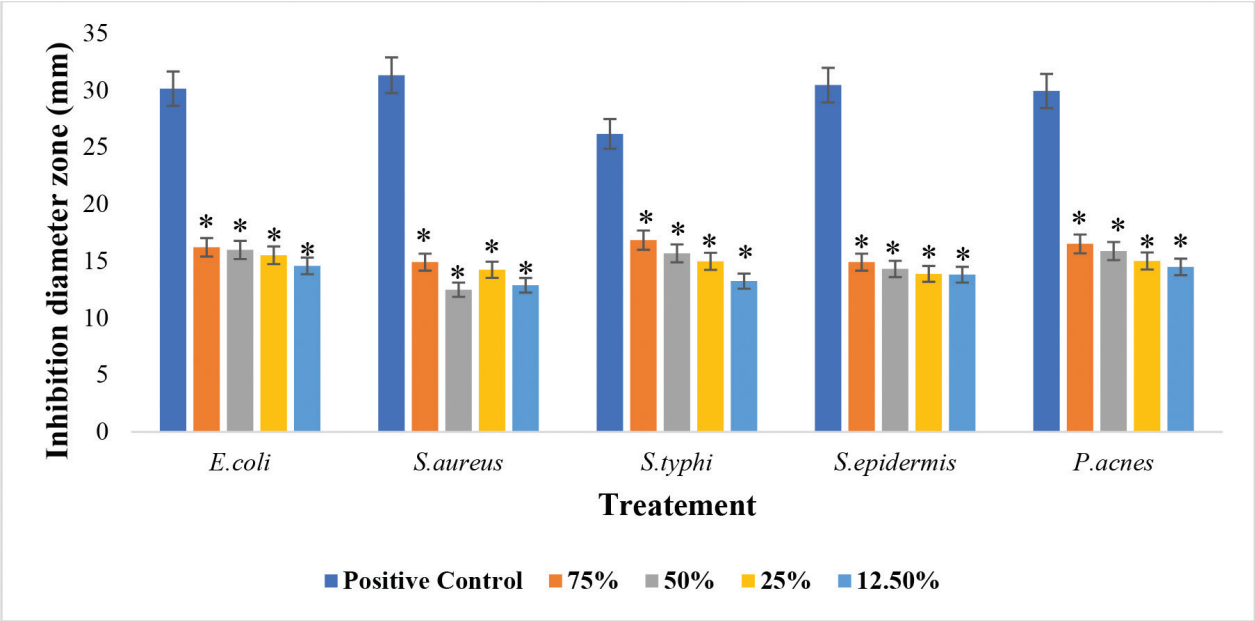

Figure 1. Nangka leave ethanol extract activity against various bacteria.

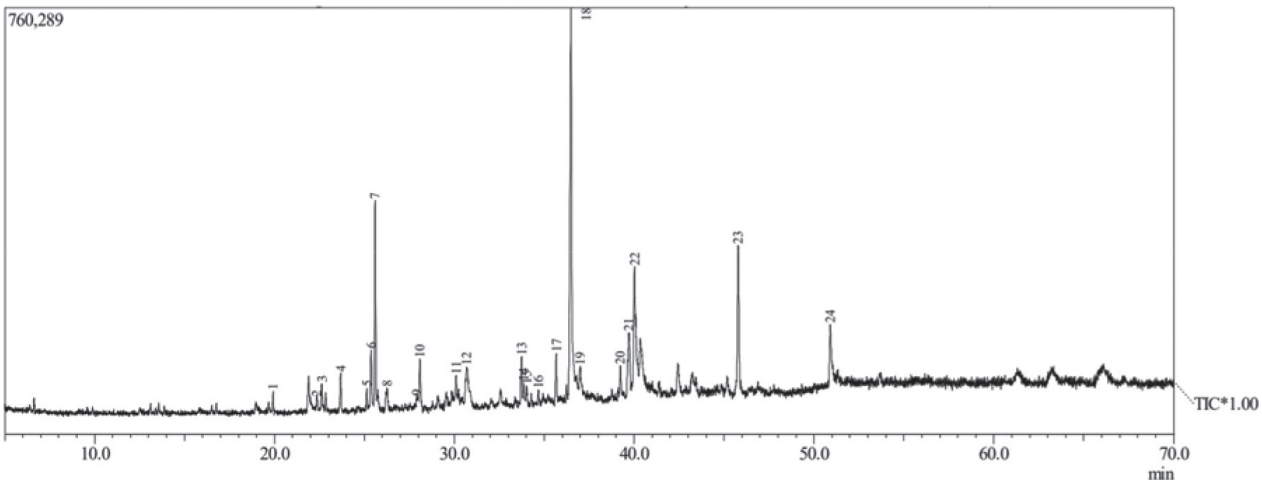

Figure 2. Chromatogram results of nangka leaves ethanol extract.

Table 2. Antibacterial inhibition of nangka leaves ethanol extract.

No.	Types of bacterials	Positive control	Concentration variation			
			75%	50%	25%	12.5%
1.	*E. coli*	30.12± 0.42	16.20±0.76*	15.97±0.67*	15.50±0.66*	14.57±0.35*
2.	*S. aureus*	31.30± 0.92	14.90±0.20*	14.48±0.50*	14.23±0.38*	12.87±0.35*
3.	*S. typhi*	26.15± 0.70	16.83±0.75*	15.67±0.60*	14.97±0.51*	13.23±0.87*
4.	*S. epidermidis*	30.43± 0.45	14.90±0.52*	14.30±0.36*	13.87±0.31*	13.80±0.17*
5.	*P. acnes*	29.92± 0.41	16.50±0.52*	15.87±0.45*	15.00±0.36*	14.48±0.50*

Data are presented in mean ± SD mm, n = 3; *p <0,05.

The antibacterial activity of the ethanol extract of nangka leaves observed for each concentration did not give a significant difference in inhibition between concentrations. The highest antibacterial activity of nangka leaves ethanol extract was found at a concentration of 75% for each of *E.coli*, *S.aureus*, *S.typhi*, *S.epidermidis* and *P. acnes* bacteria, namely 16.20±0.76 mm, 14.90±0.20 mm, 16.83±0.75 mm, 14.90±0.52 mm and 16.50±0.52 mm. The lowest activity as antibacterial was at a concentration of 12%, namely 14.57±0.35 mm, 12.87±0.35 mm, 13.23±0.87 mm, 13.80±0.17 mm and 14.48±0.50 mm. The ability of nangka leaves ethanol extract showed almost

Table 3. Compound content based on GC-MS analysis in ethanol extract of nangka leaves.

Peak	Retention time (minute)	Formula	Compounds	Abundance (%)
1	19.924	$C_{10}H_{22}$	Octane, 2,7-dimethyl	0.78
2	22.333	$C_{15}H_{24}$	Germacrene	0.82
3	22.630	$C_{12}H_{24}$	1-Dodecene	1.13
4	23.675	$C_{15}H_{24}$	trans-Caryophyllene	2.02
5	25.130	$C_{15}H_{24}$	alpha-amorphene	1.05
6	25.374	$C_{15}H_{26}O$	Farnesol	3.46
7	25.597	$C_{12}H_{26}$	Undecane	9.13
8	26.243	$C_{15}H_{24}$	γ-Cadinene	2.22
9	27.900	$C_{10}H_{18}O_2$	3,7-Dimethyl-octa-1,7-dien-3,6-diol	0.77
10	28.094	$C_{15}H_{24}O$	(-)-Caryophyllene oxide	2.80
11	30.102	$C_{11}H_{22}$	1-Undecene	1.38
12	30.676	$C_{15}H_{24}O$	(-)-Caryophyllene oxide	2.97
13	33.739	$C_{16}H_{30}O_2$	11-tetradecen-1-ol, acetate, (Z)-	2.74
14	33.867	$C_{18}H_{36}O$	2-Pentadecanone, 6,10,14-trimethyl	1.17
15	34.012	$C_{10}H_{14}O$	Longipinenepoxide	1.06
16	34.669	$C_{16}H_{30}O_2$	11-Tetradecen-1-ol, acetate, (Z)	0.76
17	35.668	$C_{17}H_{34}O_2$	Methyl 14-methyl-pentadecanoate	2.85
18	36.489	$C_{18}H_{36}O_2$	Octadecanoic acid	29.91
19	37.010	$C_{22}H_{36}O_2$	Sandaracopimar-15-ene-8 beta-yl-acetate	1.40
20	39.236	$C_{19}H_{36}O_2$	6-Octadecenoic acid, methyl ester, (Z)	1.40
21	39.709	$C_{20}H_{38}O_2$	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans	5.38
22	40.017	$C_{18}H_{34}O_2$	9-Octadecenoic acid (Z)	11.31
23	45.787	$C_{20}H_{26}O_2$	Totarol-5-en-7-on	9.78
24	50.901	$C_{18}H_{35}NO$	9-Octadecenamide, (Z)	3.71

the same activity on both gram-positive and gram-negative bacteria. Chlororamphenicol was used as a positive control because it is an antibacterial compound that has a broad spectrum, there is significance of each concentration of nangka leaves ethanol extract to the positive control ($p<0.05$).

The results of the GC-MS analysis on the ethanolic extract of nangka leaves obtained 24 peaks of compounds. The highest peak was at retention time of 36.489 minutes with a concentration of 29.91% indicating octadecanoic acid, followed by peak 22 at retention time of 40.017 minutes with a concentration of 11.31% indicating 9-octadecanoid acid (z). A clear description of the results of the GC-MS analysis is in Fig. 2, Table 3. Alleged compounds from the analysis that have potential as anti-bacterial are trans-Caryophyllene and γ-Cadinene.

Conclusions

The ethanolic extract of nangka leaves (*Artocarpus heterophyllus* Lam) had strong activity potential against various types of bacteria, both gram-negative and gram-positive bacteria and contains various bioactive compounds with the highest content of octadecanoic acid.

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