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Research Article

Phytochemical Properties, Antioxidant, and Cytotoxicity Activity of Knobweed (*Hyptis capitata*) from South Sulawesi, Indonesia

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ABSTRACT

Hyptis capitata Jacq. known as Sumambu plants in Sulawesi, has phytopharmaceutical importance. *H. capitata* extracts were evaluated for their phytochemical properties, antioxidant activity, and cytotoxicity. Using the maceration yielded five types of extracts: root chloroform (RC), root methanol (RM), leaf chloroform (LC), leaf methanol (LM), and leaf ethanol (LE). Phytochemical properties were identified by qualifying procedure and digital image analysis for quantifying Red-Green-Blue (RGB) percentage and hex colour code. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was used to determine the half-maximal inhibitory concentration (IC_{50}). Cytotoxicity screening of each extract was performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against HeLa and 4T1 cells. Gas Chromatography-Mass Spectrometry (GC-MS) assay was used to identify the phytochemical compounds of the extracts with the most promising potential. Alkaloids were the major constituents of the phytochemicals of RC, RM, LE, LC, and LM. RM and LM have potency and weak free radical scavenging activities, with IC_{50} value 31.08 and 58.03 µg/mL, respectively. The IC_{50} of RC and RM against HeLa cells were 84.21 ± 0.63 and 172.10 ± 02.90 µg/mL, respectively. Meanwhile, the cytotoxicity of RC and RM against 4T1 cells were 86.42 ± 0.80 and 182.82 ± 7.00 µg/mL, respectively. It means RC and RM exhibit a moderate level of cytotoxicity in both HeLa and 4T1 cells. LM shows moderate cytotoxicity, but it is limited to 4T1 cells with an IC_{50} value of 181.86 ± 12.68 µg/mL. The cytotoxicity level of extracts was lower than doxorubicin. Campesterol, ferruginol, stigmasterol, cis-13-octadecenoic acid methyl esters, and methyl palmitate were predicted to play a role in the antioxidant activity and cytotoxicity of RC, RM, and LM. RC, RM, and LM possess the potential for development as anticancer agents. Moreover, RM shows promise as an antioxidant due to its notable radical scavenging activity. Further research is required to explore the cytotoxic effects of RC, RM, and LM on normal cells and to assess their toxicity in experimental animals.

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INTRODUCTION

Knobweed (*Hyptis capitata* Jacq.) of the Lamiaceae family was not only native to its habitat in Central and South America but also has become

naturalised in subtropical and tropical regions of Asia and Australia (Lohr et al. 2016; Sumitha & Mini 2019; John et al. 2022). This plant has an ethnobotanical history that has been used in at least 16 countries in America, Europe, and Asia. When knobweed is applied as traditional medicine, the preparation method that is widely used is decoction (To'bungan et al. 2022a). In Indonesia, especially Sulawesi, *H. capitata* known as Sumambu or Sualang plants demonstrated anti-inflammatory properties (Audina et al. 2018; To'bungan et al. 2022a). In ethnopharmacology studies, *H. capitata* plants were found to be useful in treating diabetes mellitus, especially in Banggai, Central Sulawesi, where whole plants were used as one of ingredients in a traditional medicine preparation called jamu (Hartanti & Budipramana 2020). In a report by John et al. (2022), *H. capitata* was described as a traditional medicine used by local tribes for the treatment of asthma and fever. The leaves of this plant are also used by the Suku Anak Dalam tribe to treat wounds, both open wounds and internal wounds (Rupa et al. 2017). The pharmacological importances of this genus are for the treatment of respiratory ailments, nasal congestion, fever, liver disorders, gastrointestinal disorders, skin infections, and even human immunodeficiency virus is known (John et al. 2022). Based on this properties, *H. capitata* has been recognised as an ethnomedicinally important plant species, mainly due to its antiviral, cytotoxic, antioxidant, hepatoprotective, antimicrobial, anticancer, and anti-inflammatory properties (John et al. 2022; Sumitha et al. 2022).

Phytochemical analysis revealed that many bioactive compounds were found in this genus (John et al. 2022). *H. capitata* leaves have secretory structures forming glandular trichomes and idioblast cells (Rupa et al. 2017). The glandular trichomes contain terpenoids and alkaloids, whereas, the idioblast cells contain lipophilic compounds (Rupa et al. 2017). Terpenoid compounds such as limonene, eugenol, farnesol isomer A, d-nerolidol, coumarin, and neophytadiene could act as antimicrobial agents (Rupa et al. 2017). The composition of phytochemical compounds, especially terpenes, terpenoids, lignans, and flavonoids, varies widely among the species of *Hyptis*, depending on the geographical location, genetic, and climatic factors (John et al. 2022).

Previous study by To'bungan et al. (2022b) reported that the root chloroform extract of *H. capitata* has moderate cytotoxicity (IC_{50} 21-200 µg/mL) against T47D (34.90 ± 4.7 µg/mL) and WiDr (44.65 ± 12.07 µg/mL) cancer cells was. Based on its high cytotoxicity (13.8 ± 0.65 µg/mL), selectivity to T47D cells (3.71), and ability to induce apoptosis and antimetastatic, To'bungan et al. (2022b) concluded that the F2 fraction of *H. capitata* root chloroform extract has the highest potential as anticancer drug. The anticancer activity of the root chloroform extract was related to ferruginol, campesterol, and stigmasterol, which were able to induce apoptosis and inhibit cell migration (To'bungan et al. 2022b). *H. capitata* root methanolic extract has also been reported to have antiproliferative properties and the ability to induce apoptosis on WiDr cells associated with the phytochemical compounds chatecol, 9-hexadecanoic acid, hexadecanoic acid ethyl ester, methyl stearate, ferruginol, retinoic acid, campesterol, stigmasterol, and γ-sitosterol (To'bungan 2023). Acute toxicity and cytotoxicity of ethanolic leaf extract containing neophytadiene; hexadecanoic acid, methyl ester; hexadecanoic acid, ethyl ester; heptadecanoic acid, 16- methyl, methyl ester; phytol; ɤ-sitosterol; docosanoic acid, ethyl ester; and squalene were reported by To'bungan et al. (2022c).

No prior studies have been conducted to assess the cytotoxic effect of *H. capitata*, on cervical cancer cells (HeLa) and breast cancer cells (4T1). HeLa cells are cancer cells that are commonly used as a research

model for cervical cancer. Meanwhile, 4T1 cells was chosen as one of the cancer models, because it is more aggressive and can spontaneously metastasize from primary tumour to multiple distant (Kaur et al. 2012). Extract treatment that can inhibit cell viability will be a solution of metastatic breast cancers. Furthermore, the antioxidant activity of *H. capitata*, which naturally grows around Tana Lili, North Luwu, South Sulawesi, has not yet been investigated. Phytochemical compounds contained in *H. capitata* and thought to be involved in biological activity were traced using qualitative phytochemical tests and GC-MS (gas chromatography-mass spectrometry). Hence, the purpose of this study was to examine the anticancer potential of *H. capitata* in HeLa and 4T1 cells, along with its antioxidant activity, in order to lay the groundwork for its development as a cancer-fighting agent or novel source of natural antioxidants in the future.

MATERIALS AND METHODS

H. capitata taxonomic identification, harvesting and extraction

Taxonomic identification

H. capitata was collected by selective sampling from the Center for the Implementation of Agricultural Standards, Tana Lili, Luwu Utara, South Sulawesi, Indonesia (Figure 1). Identification was performed under the certification number 014535/S.Tb/III/2019 (To'bungan et al. 2022b) at the Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada, DIY. *H. capitata* was identified as the genus *Hyptis* with the NCBI reference code: txid204124 (Schoch et al. 2020).

Harvesting

H. capitata root and leaf parts were harvesting in the morning and prepared by washing and grouping. The roots and leaves were covered with black cloth and dried under the sun until a constant dry weight was obtained. Powder was prepared by grinding the dried roots and leaves. The resulting powder was sieved through a 44-mesh sieve. The sieved root and leaf powder were stored at room temperature (± 25℃) in containers filled with food grade silica gel.

Figure 1. Root (**a**), leaf (**b**), and shoot (**c**) parts of *H. capitata*. Personal records of To'bungan (2022). *H. capitata* was collected from its natural habitat in Tana Lili, Luwu Utara, South Sulawesi, Indonesia (2°36'46.6"S 120°35'28.5" E). Map of Indonesia and Southeast Asia generated by Mahardhika (2023).

Extraction

Extraction was performed by the maceration method, where the leaf and root mesh powder were treated with physical and chemical processing (solvent). Ratio of leaf and root powder with solvent is 1:5. The solvent-powder solution was prepared by weighing 100 g leaf and root powder. Absolute chloroform, absolute methanol, and absolute ethanol were the solvents used to prepare leaf extracts. Whereas, the solvents used to prepare root extracts were absolute chloroform and absolute methanol. Merck KGaA EMSURE®, Germany produced the solvents used in extraction. Different solvents were used to determine their phytoextraction efficacy. The maceration process was carried out for 48 h. It was stirred occasionally throughout the process. Each solution was filtered through a Whatman® Paper Filter 42 and dried in a porcelain cup at room temperature (± 25°C) until the evaporation was complete. The whole process resulted in root chloroform (RC), root methanol (RM), leaf chloroform (LC, leaf methanol (LM), and leaf ethanol (LE) extracts.

Phytochemical screening tests

Alkaloids

A stock solution was prepared by weighing 30 mg of each extract and dissolving it in 10 mL absolute chloroform. 300 µL ammonia was added to the stock solution and the mixture was homogenized. The homogeneous stock solution was divided into three groups of 2 mL each in accordance with the reagents. To each group, 1 mL sulfuric acid 2 M was added. The mixture was homogenized and left for 5 min. Two layers were formed and the bottom half of the layer was completely discarded. Groups I, II, and III were then treated with 1 mL Dragendorff's (Merck KGaA, Germany), Mayer's (Merck KGaA, Germany), and Wagner's (Merck KGaA, Germany) reagents, respectively. The formation of red-brown-orange (Dragendorff's), white (Mayer's), and brown (Wagner's) precipitates has been observed and documented as indicative of the presence of alkaloids.

Saponins

Extracts were weighed to 30 mg and dissolved in 5 mL aquadest. The solution was then homogenized. Foam formation was observed. The presence of saponins was indicated by a foam measuring 1-3 cm and lasting 15 min.

Flavonoids

Extracts were weighed to 0.1 g and dissolved in 5 mL 30% methanol. A stirring hot plate (Thermo Scientific™ Cimarec+™) was used to heat the solution at 250℃ for 5 min. To each of the solutions, 250 µL concentrated sulfuric acid 2 N was added. The red coloration indicates that flavonoids are present.

Tannins

The solution from the saponins test was heated to 100℃ using the Memmert® Waterbath WNB14. 250 µL $FeCl_3$ 10% was added to the solution. The color change to blue or turquoise black was observed to indicate the presence of tannins in the extracts.

Triterpenes and Steroids

Extracts were weighed to 0.1 g and transferred to a drop plate. To the extracts, 150 µL anhydrous sodium acetate (EMSURE®, Canada) and 50 µL concentrated sulfuric acid 2 N were added. The color change to red and green, respectively, was observed as an indicator of the presence of

triterpenes and steroids.

Antioxidant activity

DPPH radical scavenging assay (Abdulrahman et al. 2019)

Determination of maximum wavelength (λ) and absorbance (A)

8 mg DPPH (Sigma Adrich, Singapore) was dissolved in 200 mL EMSURE® absolute ethanol to prepare 40 µg/mL DPPH. The homogeneous DPPH reagent solution was incubated for 30 min at room temperature (± 25°C) in dark bottles covered with aluminum foil. EMSURE® absolute ethanol (AE) was used as a blank solution for the assay. A UV-Vis spectrophotometer (Thermo Fisher Scientific™ Genesys 10S UV-Vis, SN: 2L9L1019203) was used to determine the maximum wavelength (λ_{max}) and absorbance (A_{max}) of the DPPH solution. The entire procedure was performed in darkness. Light contamination was carefully avoided. The statistical design was three replications, repeated three times (each extract concentration tested was made in triplicate and also repeated three times).

Standard solutions

As a standard and positive control, ascorbic acid (AA) was used. 4 mg EMSURE® ascorbic acid was dissolved in 20 mL EMSURE® absolute ethanol to prepare a 200-ppm stock solution. The dilution series included five concentrations: 2, 4, 6, 8, and 10 µg/mL. 1 mL AA from each concentration was stored in the dark vials. To the AA solution, a 4 mL DPPH solution was added. Incubation was performed without light for 30 min at room temperature (±25°C). The absorbance of AA was measured using a UV-Vis spectrophotometer (Thermo Fisher™ Genesys 10S UV-Vis, SN: 2L9L1019203) at the maximum wavelength (λ_{max}) of the DPPH solution. The statistical design consisted of three replications, repeated three times for each concentration.

Extract solutions

The 200 µg/mL stock solution was prepared by dissolving 4 mg in 20 mL of EMSURE® absolute ethanol of the extracts consisting of RC, RM, LE, LC, and LM. The dilution series included five concentrations: 12.5, 25, 50, 100, and 200 µg/mL. To each stock solution of the respective extracts, 1 mL was stored in dark bottles and 4 mL DPPH solution was added. The incubation process was carried out in the absence of light for 30 min at room temperature (± 25°C). Absorbance of each extract was measured using a UV-Vis spectrophotometer (Thermo Fisher Scientific™ Genesys 10S UV-Vis, SN: 2L9L1019203) at maximum wavelength (λ_{max}) of DPPH solution (517 nm). For each concentration of the respective extracts, the statistical design consisted of three replicates repeated three times (each extract concentration tested was made in triplicate and also repeated three times).

In vitro cytotoxicity screening in HeLa and 4T1 cancer cells

HeLa and 4T1 cancer cells were obtained from the Laboratory of Parasitology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta. HeLa and 4T1 cancer cells were cultured in complete Roswell Park Memorial Institute 1640 (RPMI) medium (Gibco™, Canada), with 96 well plate at a density of 11 x 10^3 cells/well and 8 x 10^3 cells/well, respectively. The assay procedure was in accordance with the previously published protocol by To'bungan et al. (2022b). Ethics Committee of the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Ma-

da,Yogyakarta-Dr. Sardjito General Hospital certified the use of cancer cells with ref. KE/FK/0011/EC/2023 (validation number: 63b4ee136addb).

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS (Thermo Scientific Trace 1310 Gas Chromatograph-ISQ LT Single Quadropole Mass Spectrometry) analysis was performed at the Laboratory of Integrated Research and Testing, Universitas Gadjah Mada, Yogyakarta, according to the procedure previously published by To'bungan et al. (2022b). The chromatogram, retention time (min), chemical equation, relative area (%), molecular weight, and phytochemical compounds of each extract were obtained (Supp. File 3). The retention time (min) is represented by the x-axis of the chromatograms, while the y-axis is explained as the relative area (%). The retention time is the time taken for the analytes to pass through the columns and reach the mass spectrometer detector. Relative area (%) is based on the number of counts taken by the mass spectrometer detector at the point of retention. Relative area (%) uses the target component peak area as a proportion of the total area of all detected peaks. Relative area (%) is used to approximate the concentration of phytochemical compounds identified during retention time. The extracts further analyzed by GC-MS were selected based on the lowest IC_{50} value of DPPH radical scavenging and cytotoxicity assay. The phytochemical compounds from each extract were identified using the National Institute of Standards and Technology (NIST) library similarity index and the NIST Chemistry WebBook, SRD 69 (https://doi.org/10.18434/T4D303). Phytochemical compounds were cross-referenced with National Library of Medicine (NLM)/NCBI PubChem and various credible sources for relevant therapeutic potential.

Statistical and digital image analysis

Absorbance of blank, DPPH, standard, and extracts were recorded. The following formula was used to calculate the absorbance of extracts~standard [1.1~1.2] and control [2]:

[1.1] Absorbance of extracts=Absorbance of extracts - Absorbance of blank

[1.2]Absorbance of standard=Absorbance of standard - Absorbance of blank

[2]Absorbance of control=Absorbance of DPPH - Absorbance of blank

The inhibition of extracts~standard [3.1~3.2] was calculated using the following formula:

$$[3.1] \quad \%\ \text{Inhibition} = \left[\frac{(\text{Absorbance of control} - \text{Absorbance of extracts})}{\text{Absorbance of control}}\right] \times 100\%$$

$$[3.2] \quad \%\ \text{Inhibition} = \left[\frac{(\text{Absorbance of control} - \text{Absorbance of blank})}{\text{Absorbance of control}}\right] \times 100\%$$

John et al. (2022)

The linear regression between $\log_e x$ concentration (X-axis) of extracts and standard with their respective inhibition percentage (Y-axis) was used to calculate the half-maximal inhibitory concentration (IC_{50}) of extracts~standard. To determine the reliability of the results, the r^2 value greater than 0.7 was used. The equation obtained from the linear regression [4] was used to calculate the IC_{50} as follows:

[4] $y = 50$

$x = IC50$

$\ln IC50 = \frac{(y-b)}{a}$

$IC50\ (\mu g/ml) = e^x$

Regression and data presentation were performed using the algorithm (Supp. File 2) in the RStudio/2023.03.1+446 ©2009-2023 Posit Software, PBC "Cherry Blossom" release (6e31ffc3, 2023-05-09) for Windows. IBM® SPSS® Statistics v. 21 was used to analyze the data for one-way analysis of variance (ANOVA) and Tukey's HSD *post hoc* analysis.

IC_{50} of cytotoxicity test obtained from linear regression analysis of log concentration. The concentration that inhibited cell growth by 50% (IC_{50}) was determined from the data obtained. In addition, the data were analysed using IBM® SPSS® Statistics v. 21 for one-way analysis of variance (ANOVA) followed by Tukey's HSD *post hoc* analysis.

Digital image analysis was performed in the environment of RStudio/2023.03.1+446 ©2009-2023 Posit Software, PBC "Cherry Blossom" release (6e31ffc3, 2023-05-09) for Windows using the color quantification algorithm (Supp. File 1). For further cross-referencing against a web-based database, digital image analysis was performed to translate the qualitative characteristics of each image into quantifiable measurements in the form of RGB percentage and hex color code. The highest value of the RGB color space and its corresponding hex color code were cross-referenced using a web-based colour database.

RESULTS AND DISCUSSION

Results

Phytochemical properties

Phytochemical compounds play a role in many biological activities, including antioxidant and cytotoxic (anticancer) activities. A qualitative search for phytochemical compounds provides a general description of the group of compounds predicted to be contained in each extract tested (Table 1). Subsequent colour quantification and cross-referencing confirmed the presence of alkaloids in the RC extract based on Dragendorff's and Mayer's reagents. Only Dragendorff's indicated alkaloids in the RM, LE, LC, and LM extracts. Due to its selectivity and specificity (Raal et al. 2020), Dragendorff's was the primary reagent for the identification of alkaloids. Dragendorff's (potassium bismuth iodide) consists of basic bismuth nitrate ($Bi(NO_3)_3$), tartaric acid, and potassium iodide (KI) (Raal et al. 2020). Alkaloid testing with Dragendorff's reagent used nitrogen to form coordinate covalent bonds with metal ion K^+, resulting in potassium alkaloid precipitates (Parbuntari et al. 2018; Sabdoningrum et al. 2021). When identifying with Dragendorff's reagent, the risk of false-positive results usually requires the use of other reagents. Raal et al. (2020) stated that the presence of other plant constituents, such as purines, proteins, betaines, and ammonium salts, might also yield positive results, so a positive result might not always indicate the presence of alkaloids. In addition, Dragendorff's reagent was not precipitate caffeine, strychnine, and brucine (Raal et al. 2020). To confirm the positive results in this study, Mayer's and Wagner's reagents were used in addition to Dragendorff's reagent. As a result, only RC extract was positive for both Dragendorff's and Mayer's reagents. Alkaloids could be confirmed as a constituent in the extracts of the *H. capitata*. However, possible false positives could be suspected from the negative results with Wagner's and Mayer's reagents.

Saponins were present in RM and LE extracts. Saponins are structurally complex steroidal and triterpenoidal amphiphatic glycosides that are widely produced by plants and have surfactant properties, forming a stable soap-like foam when shaken in aqueous solution (Shi et al. 2004; Faizal & Geelen 2013; Mugford & Osbourn 2013; Parbuntari et al. 2018; Rai et al. 2021; Sabdoningrum et al. 2021). The term saponin defines a

Table 1. Phytochemical properties of extracts.

Extracts	Alkaloid			Saponin	Flavonoid	Tannin	Triterpene/Steroid
	Dragendorff	Mayer	Wagner				
RC	(+)	(+)	(-)	(-)	(+)	(-)	(+ triterpenes)
RM	(+)	(-)	(-)	(+)	(+)	(+)	(+ triterpenes)
LE	(+)	(-)	(-)	(+)	(-)	(+)	(+ steroids)
LC	(+)	(-)	(-)	(-)	(-)	(-)	(+ steroids)
LM	(+)	(-)	(-)	(-)	(-)	(-)	(+ steroids)

Note: See Supp. File 1 for detailed color quantification procedures and results. Positive (+) and negative (-) annotations were validated by cross-referencing the highest RGB percentage and hex color code with a web-based color database. *H. capitata* extracts: root chloroform (RC), root methanol (RM), leaf ethanol (LE), leaf chloroform (LC), and leaf methanol (LM). Phytochemical screening was performed to determine alkaloids, saponins, flavonoids, tannins, and triterpenes/steroids in RC, RM, LE, LC, and LM extracts of *H. capitata* (Table 1).

group of high molecular weight glycosides that are composed of a glycan moiety linked to an aglycon, which is also referred to as genin or sapogenin (Faizal & Geelen 2013; Rai et al. 2021). Triterpenes and sterols both originate from the mevalonate pathway with 2,3-oxidosqualene as a common precursor (Mugford & Osbourn 2013).

Tannins were present in RM and LE extracts. Plant tannins are a unique group of phenolic metabolites with a relatively high molecular weight ranging from 500 to 3000 Da that have the ability to form strong complexes with carbohydrates and proteins (Serrano et al. 2009; Singh & Kumar 2019). Tannins are composed of an aggregation of complex phytochemical compounds and secondary metabolites, such as hydroxyl groups.

Flavonoids were present in RC and RM extracts. Flavonoids are an important class of natural products. In particular, they belong to a class of plant secondary metabolites with a polyphenolic structure of benzo-□-pyrone (Kumar & Pandey 2013; Panche et al. 2016). Flavonoids are synthesised through the phenylpropanoid pathway, with the conversion of phenylalanine to 4-coumaroyl-CoA, which is ultimately entered into the flavonoid biosynthetic pathway (Ferreyra et al. 2012; Kumar & Pandey 2013).

Triterpenes were present in RC and RM extracts. Among these secondary metabolites, terpenes constitute a major class further subdivided into monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, and tetraterpenoids (Darshani et al. 2022). Triterpenes are 30-carbon secondary metabolites formed by the combination of six isoprene units in accordance with Ruzicka's isoprene rule (Darshani et al. 2022).

LE, LC, and LM extracts were found to contain steroids. Steroids which are derived from the terpenoid building block isopentenyl pyrophosphate are a subclass of terpenoids that contain a characteristic arrangement of four cycloalkane rings that are connected to each other (Tong 2013; Patel & Savjani 2015). Terpenoids are a large class of oxygenate terpene analogues.

Antioxidant activity

The maximum wavelength (λ_{max}) and the incubation time (T) corresponding to the maximum absorbance (A_{max}) of the 40 µg/mL DPPH solution were found to be 517 nm and 30 min, respectively. The assay was continued by following the obtained λ_{max} and incubation time (T) of the DPPH solution to determine the absorbance of the standard (AA) and extracts (RC, RM, LE, LC, and LM) at their respective concentrations. Radical scavenging activity is determined based on the high and low absorption values of the extracts tested.

Log$_e$x concentration and % inhibition

Absorbance is inversely proportional to % inhibition. The % inhibition of 48.002 ± 1.82(4%) at the concentration of 10 ppm confirms the highest value of AA. As the concentration increases, the % inhibition increases steadily. A significant (p<0.05) difference in % inhibition is observed among the concentrations based on *post hoc* analysis. The extracts also showed a similar trend of % inhibition. For all extracts, the highest % inhibition was observed at 200 µg/mL (Table 2). The % inhibition of RC, RM, LE, LC, and LM were 28.92 ± 4.97(17%), 93.791±1.25(1%), 41.06±0.37(1%), 37.99±0.59(2%), and 70.16±1.44(2%), respectively. Confirming the absorbance-based assumption, the highest % inhibition was observed in RM extract, followed by LM, LE, LC, and RC. *Post hoc* analysis showed significant (p<0.05) difference between % inhibition of RC, RM, LC, and LM. However, there was an insignificant difference in the % inhibition of LC and LE at the concentration of 200 µg/mL. Further confirming the absorbance-based assumption, a significant difference (p < 0.05) between concentrations was observed in RM extract.

Half-maximal inhibitory concentration (IC_{50})

Half-maximal inhibitory concentration (IC_{50}) is related to extract radical scavenging activity. The radical scavenging activity can be used to interpret the antioxidant activity. DPPH has been widely used as an *in vitro* assay for the antioxidant activity of plant extracts (Kiziltas et al. 2022; Klomsakul et al. 2022; Tariq et al. 2022; Vidhya et al. 2022). IC_{50} was calculated using the formula obtained from the linear regression between log_ex concentration and % inhibition. A series of optimisation was carried out based on the reliability of r^2 > 0.7. Based on the IC_{50} value, RM extract followed by LM had the minimum concentration for inhibiting at least half (50%) of the initial DPPH concentration (Table 3). The concentration and composition of phytochemical compounds may be responsible for the antioxidant activity of RM extract. As a standard, the IC_{50} value of AA showed the highest free radical scavenging activity. IC_{50} value of RM extract is classified as potent, while LM and AA are classified as weak and potent, respectively. *Post hoc* analysis showed the significant (p < 0.05) difference between each extract. Insignificant differences were observed for RC, LE, and LC. Meanwhile, RM and LM are significantly different from each other and from RC, LE, and LC.

Cytotoxicity assay in HeLa and 4T1 cells

HeLa and 4T1 cancer cells were used to evaluate the cytotoxicity of the extracts. DMSO and DOX were used as negative and positive controls, respectively. Cytotoxicity is represented by the IC_{50} value, which describes the minimum extract concentration that inhibits at least half (50%) of the viability of cancer cells (Nordin et al. 2018). The most potent extract in terms of cytotoxicity IC_{50} was RC, followed by RM extract (Table 4). Treatment with either RC or RM extract showed insignificant differences (p < 0.05) in both HeLa and 4T1 cells. In the 4T1 cells, the treatments led to more diverse result. LM extract presented countable cytotoxicity in 4T1 cancer cells. There was also a significant difference (p < 0.05) between the LC and the three extracts of RC, LM, and RM in the 4T1 cancer cell. The cytotoxicity of RC and RM extracts in HeLa cancer cells was in the category of moderate cytotoxicity. Meanwhile, RC, LM, and RM expressed moderate cytotoxicity in 4T1 cancer cells. In the 4T1 cells, the treatment of LC extract was categorised as non-toxic. To'bungan et al. (2022b) reported a moderate cytotoxicity of *H. capitata* root chloroform extract against T47D (34.90±4.7 µg/mL) and WiDR

Table 2. Radical scavenging inhibition of RC, RM, LE, LC, and LM.

Concentration (µg/mL)	% Inhibition ± SD (% CV)				
	RC	RM	LE	LC	LM
200	28.92±4.97(17%)	93.791±1.25(1%)	41.06±0.37(1%)	37.99±0.59(2%)	70.16±1.44(2%)
100	25.98±6.11(24%)	86.76±1.38(2%)	37.43±0.61(2%)	35.18±0.19(1%)	53.64±0.68(1%)
50	24.48±5.38(22%)	56.54±0.47(1%)	31.64±0.14(0%)	31.42±1.86(6%)	44.14±1.32(3%)
25	22.83±6.38(28%)	41.49±1.16(3%)	28.19±0.59(2%)	29.34±2.23(8%)	37.49±1.71(5%)
12.50	21.33±6.68(31%)	30.41±2.89(10%)	27.21±1.102(4%)	29.74±0.65(2%)	35.43±1.89(5%)

Table 3. Antioxidant IC_{50} from samples.

Samples	RC	RM	LE	LC	LM	AA
$\bar{x}$ IC_{50} (µg/mL)	$>100^{d}$	31.08^{b}	$>100^{d}$	$>100^{d}$	58.03^{c}	13.36^{a}
±SD	>100	1.035	91.97	>100	4.16	1.03
CV%	32	3	8	24	7	8

Note: One-way ANOVA followed by *post hoc* Tukey HSD produced mean ($\bar{x}$) from three replications. Superscripts with different letter described the significance ($p<0.05$) between IC_{50} (µg/mL) samples. SD = Standard Deviation; CV% = Coefficient of Variation. Value above a thousand written as >100. Ascorbic acid (AA) as standard and positive control. *H. capitata* extracts: root chloroform (RC), root methanol (RM), leaf ethanol (LE), leaf chloroform (LC), and leaf methanol (LM). IC_{50} value classification: <10 µg/mL (very potent), 10-50 µg/mL (potent), 50-100 µg/mL (weak), 100-250 µg/mL (very weak), and >250 µg/mL (inactive) (Reviana et al. 2021).

Table 4. Cytotoxicity IC_{50} against cervical cancer cells (HeLa) and breast cancer cells (4T1).

Treatments	$\bar{x}$ IC_{50} ± SD (µg/mL)	
	HeLa	4T1
RC	84.21 ± 0.63^{b}	86.42 ± 0.80^{b}
LC	>1000	548.33 ± 98.44^{c}
LM	>1000	$181,86 \pm 12,68^{b}$
RM	172.10 ± 2.90^{b}	182.82 ± 7.00^{b}
LE	>1000	>1000
DOX	1.22 ± 0.33^{a}	0.11 ± 0.05^{a}
DMSO	>1000	>1000

Note: One-way ANOVA followed by *post hoc* Tukey HSD produced mean ($\bar{x}$) from three replications. Superscripts with different letter described the significance ($p < 0.05$) between IC_{50} (µg/ml) of treatments. SD = Standard Deviation. Value above a thousand written as >1000. Dimethyl sulfoxide (DMSO) as negative control. Doxorubicin (DOX) as positive control. *H. capitata* extracts: root chloroform (RC), root methanol (RM), leaf ethanol (LE), leaf chloroform (LC), and leaf methanol (LM). Cytotoxicity categories based on the US National Cancer Institute (Hameed 2012; Srisawat et al. 2013): very toxic $IC_{50} \leq 20$ µg/mL, moderate/toxic IC_{50} 21–200 µg/mL, weak IC_{50} 201–500 µg/mL, and non-toxic $IC_{50} \geq 500$ µg/mL.

(44.65±12.07 µg/mL) cancer cells. The methanolic extract of the *H. capitata* root has also been reported to have anti-proliferative properties and the ability to induce apoptosis on WiDR cells by To'bungan (2023). To identify and confirm the association of phytochemical compounds in RC, RM, and LM with antioxidant activity and cytotoxicity, the investigation was continued by GC-MS assay.

GC-MS assay

The RC, RM and LM chromatograms can be seen in Figure 2-4. GC-MS detected 116, 67 and 44 peaks, respectively. The predicted phytochemical compounds in each extract are presented in Table 5-6. The detected phytochemical compounds which are predicted to have antioxidant and cytotoxic activity, include campesterol, ferruginol, stigmasterol, cis-13-octadecenoic acid methyl esters, and methyl palmitate.

Root chloroform extract

As shown in Figure 2, 116 peaks were identified. RC extract consisted of six phytochemical compounds with RA higher than 1, representing most constituents. The identified phytochemical compounds were pregn-5-en-20-one, 3-hydroxy, (3ß,17a)-, methyl retinoate, ferruginol, quassin, stigmasterol, and campesterol (Table 5).

Figure 3 shows 67 peaks identified. The RM extract was composed of six phytochemical compounds with RA higher than 2, which represented the majority of the constituents. The phytochemical compounds identified were gibberellin A44 (GA44), ferruginol, cis-13-octadecenoic acid methyl ester, methyl palmitate, stigmasterol, and quassin (Table 5).

Figure 4 shows 44 peaks identified. The LM extract was composed of six phytochemical compounds with RA higher than 3 which represented the majority of the constituents. The phytochemical compounds identified were trans-13-octadecenoic acid methyl ester, ethyl linolenate, ethyl palmitate, methyl palmitate, neophytadiene, and methyl isostearate (Table 7).

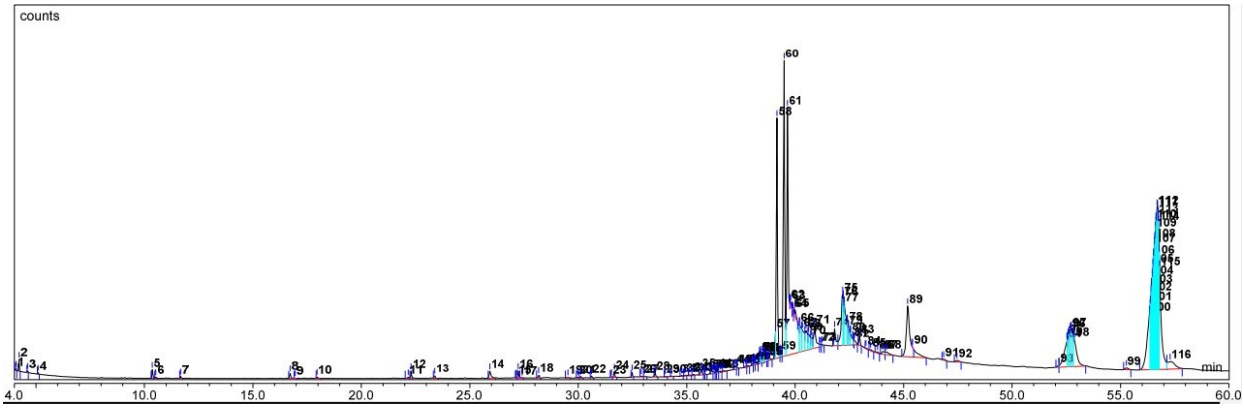

Figure 2. Chromatograms of *H. capitata* root chloroform extract (RC). GC-MS detects 116 peaks.

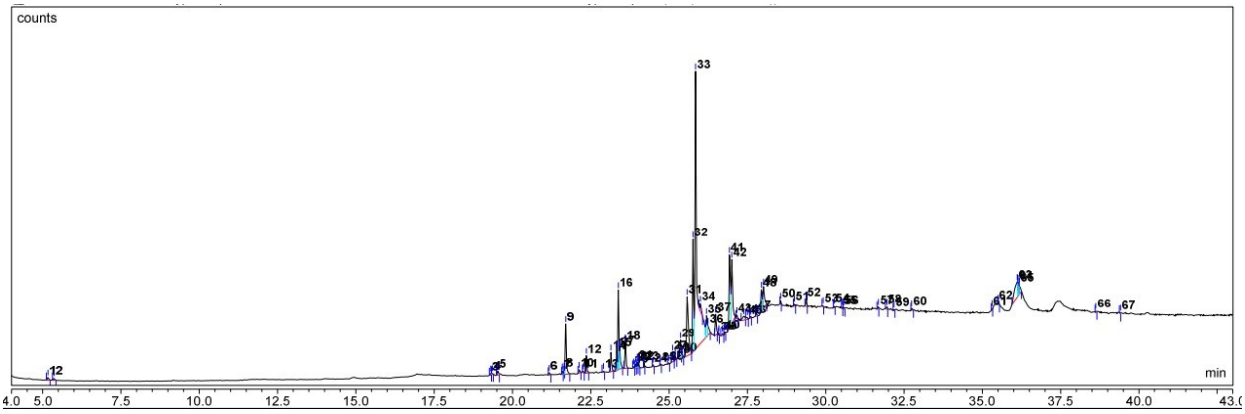

Figure 3. Chromatograms of *H. capitata* root methanol extract (RM). GC-MS detects 67 peaks.

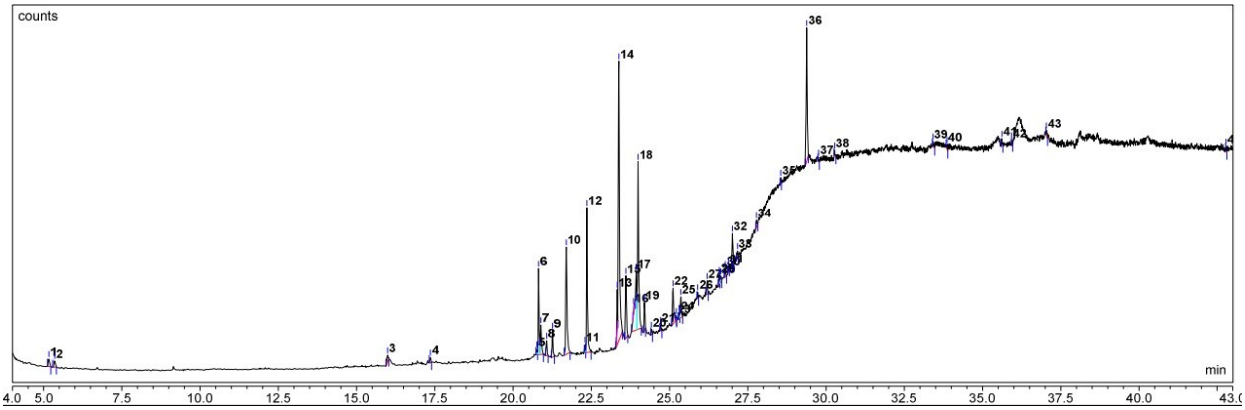

Figure 4. Chromatograms of *H. capitata* leaf methanol extract (LM). GC-MS detects 44 peaks.

Table 5. Identified phytochemical compounds of RC extract of *H. capitata*.

No.	RT (min)	Chemical formula	Phytochemical compounds	RA (%)	Bioactivities	Classifications
1	10.36	$C_{11}H_{24}$	undecane	0.14	*X*	alkanes [MeSH]
2	11.67	$C_{10}H_{22}$	decane	0.03	*X*	alkanes [MeSH]
3	16.71	$C_{15}H_{32}$	2,7,10-trimethyldodecane	0.14	*X*	alkanes [ChEBI]
4	16.95	$C_{19}H_{40}$	6-methyloctadecane	0.01	*x*	alkanes [ChEBI]
5	17.96	$C_{17}H_{36}$	2,6,10-trimethyltetradecane	0.05	*v*	fatty acids [LOTUS]
6	22.29	$C_{16}H_{34}$	hexadecane	0.13	*ox*; *bx*	alkanes [MeSH]
7	25.92	$C_{13}H_{14}O_4$	1'-acetoxychavicol acetate	0.31	*v*	alcohols; benzyl alcohols [MeSH] carboxylic ester [ChEBI]
8	27.16	$C_{26}H_{54}$	3-ethyl-5-(2-ethylbutyl) octadecane	0.01	*ox*; *bx*	alkanes [ChEBI] fatty acids [LOTUS]
9	27.23	$C_{21}H_{44}$	2,6,10,15-tetramethylheptadecane	0.16	*x*	prenol lipids; sesquiterpenoids [HMDB0062787]
10	27.42	$C_{17}H_{32}O_2$	7-methyl-z-tetradecen-1-ol acetate	0.02	*cx*	carboxylic esters [ChEBI] fatty acids [LOTUS]
11	29.53	$C_{17}H_{32}O$	13-heptadecyn-1-ol	0.04	*v*	alcohols; fatty alcohols [ChEBI]
12	30.11	$C_{17}H_{36}O$	2-methyl-1-hexadecanol	0.05	*v*	fatty acids; fatty alcohols [LOTUS]
13	31.54	$C_{25}H_{44}N_2O_5S$	2-myristynoyl pantetheine	0.01	*x*	fatty acids; n-acyl amines [LOTUS]
14	32.91	$C_{18}H_{24}O_2$	methyl 5,8,11-heptadecatriynoate	0.03	*x*	fatty acids; methyl esters [CAS 56554-57-5]
15	33.05	$C_{18}H_{36}O_2$	ethyl palmitate	0.04	*ox*	fatty acids; palmitic acids [MeSH]
16	33.55	$C_{20}H_{40}O_2$	ethanol, 2-(9-octadecenyloxy)-, (z)-	0.03	*cx*	dialkyl ethers [CAS 5353-25-3]
17	36.42	$C_{26}H_{54}$	3-ethyl-5-(2-ethylbutyl) octadecane	0.06	*ox*; *bx*	alkanes [ChEBI] fatty acids [LOTUS]
18	37.23	$C_{37}H_{76}O$	1-heptatriacontanol	0.11	*v*	fatty acids; fatty alcohols [LOTUS]
19	38.36	$C_{16}H_{30}O_2$	9-hexadecenoic acid	0.20	*v*	fatty acids [MeSH]
20	39.09	$C_{20}H_{30}O_2$	podocarpa-8,11,13-triene-7ß,13-diol, 14-isopropyl-	0.47	*cx*; *bx*	terpenoids; podocarpane diterpenoids [LOTUS]
21	39.16	$C_{20}H_{30}O$	ferruginol	6.72	*v*	terpenoids; diterpenoids (C20) [KEGG: phytochemical compounds]
22	39.49	$C_{21}H_{30}O_2$	methyl retinoate	8.78	*v*	vitamins; vitamin A [MeSH] prenol lipids; retinoids; retinoid esters [HMDB0254612]
23	39.65	$C_{21}H_{32}O_2$	pregn-5-en-20-one, 3-hydroxy-, (3ß,17a)-	16.21	*v*	alcohols; secondary alcohols; 3beta-hydroxy steroids; pregnenolone [ChEBI]

Table 5. Contd.

No.	RT (min)	Chemical formula	Phytochemical compounds	RA (%)	Bioactivities	Classifications
24	39.79	$C_{20}H_{28}O_3$	16-hydroxymethyleneandrost-5-en-3-ol-17-one	0.06	*cx*	alcohols; secondary alcohols; 3beta-hydroxy steroids; dehydroepiandrosterone [ChEBI] terpenoids; androstane steroids [LOTUS]
25	41.82	$C_{20}H_{38}O_2$	paullinic acid	0.71	*x*	fatty acids [ChEBI]
26	42.97	$C_{69}H_{134}O_6$	tribehenin	0.25	*x*	fatty acids [MeSH]
27	45.41	$C_{22}H_{28}O_6$	quassin	5.16	*x*	terpenes [MeSH]
28	52.16	$C_{26}H_{44}O_5$	ethyl iso-allocholate	0.07	*v*	terpenoids; cholane steroids [LOTUS]
29	52.69	$C_{28}H_{48}O$	campesterol	1.31	*ox*; *cx*	alcohols; secondary alcohols; 3beta-hydroxy steroids [ChEBI] terpenoids; steroids [KEGG: phytochemical compounds]
30	56.75	$C_{29}H_{48}O$	stigmasterol	2.75	*v*	alcohols; secondary alcohols; 3beta-hydroxy steroids [ChEBI] terpenoids; steroids [KEGG: phytochemical compounds]

Note: RT = retention time; RA = relative area; *ox* = antioxidant/radical scavenging activity; *bx* = antibacterial/ antimicrobial; *cx* = cytotoxicity/anticancer/antiproliferative/anticarcinogenic; *x* = no activity reported with respect to *ox*, *bx*, and *cx*; *v* = *ox*, *bx*, and *cx* activities reported. See Supp. File 3.

Table 6. Identified phytochemical compounds of RM extract of *H. capitata*.

No.	RT (min)	Chemical formula	Phytochemical compounds	RA (%)	Bioactivities	Classifications
1	5.17	$C_{27}H_{44}O_3$	1,25-dihydroxyvitamin D3	0.29	*cx*; *bx*	vitamins; cholecalciferols [MeSH]
2	21.70	$C_{17}H_{34}O_2$	methyl palmitate	3.35	*ox*; *bx*	fatty acids [MeSH]
3	22.36	$C_{18}H_{36}O_2$	ethyl palmitate	0.93	*ox*	fatty acids [MeSH]
4	23.15	$C_{20}H_{30}$	7-isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	1.22	*x*	terpenoids [LOTUS]
5	23.33	$C_{19}H_{34}O_2$	7,10-octadecadienoic acid, methyl ester	1.14	*x*	lineolic acids and derivatives [CAS 56554-24-6]
6	23.38	$C_{19}H_{36}O_2$	cis-13-octadecenoic acid, methyl ester	4.65	*cx*	fatty acids [MeSH]
7	23.43	$C_{19}H_{36}O_2$	10-octadecenoic acid, methyl ester	1.55	*ox*; *bx*	fatty acids [LOTUS]
8	23.60	$C_{19}H_{38}O_2$	methyl isostearate	1.5	*x*	fatty acids [LOTUS]
9	23.93	$C_{26}H_{44}O_5$	ethyl iso-allocholate	0.18	*v*	terpenoids; cholane steroids [LOTUS]
10	25.11	$C_{35}H_{68}O_5$	1,2-dipalmitoyl-rac-glycerol	0.54	*x*	glycerolipids [ChEBI]
11	25.36	$C_{24}H_{36}O_6$	8,14-seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3ß-methoxy-4,4-dimethyl-	1.17	*cx*	ketals [PubChem CID 550132]

Table 6. Contd.

No.	RT (min)	Chemical formula	Phytochemical compounds	RA (%)	Bioactivities	Classifications
12	25.57	$C_{20}H_{30}O$	ferruginol	7.21	*v*	terpenoids; diterpenoids (C20) [KEGG: phytochemical compounds]
13	25.76	$C_{20}H_{26}O_5$	gibberellin A44 (GA44)	7.90	*x*	isoprenoids; terpenoids; diterpenoids; gibberellins [ChEBI]
14	26.00	$C_{20}H_{28}O_3$	16-hydroxymethyleneandrost-5-en-3-ol-17-one	0.73	*cx*	alcohols; secondary alcohols; 3beta-hydroxy steroids; dehydroepiandrosterone [ChEBI] terpenoids; androstane steroids [LOTUS]
15	26.20	$C_{32}H_{48}O_6$	dodecanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5a-hydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-5-yl ester, [1aR-(1aa,2a,5ß,5aß,8aa,9a,10aa)]-	1.71	*bx*; *ox*	fatty acids; lauric acids [MeSH] fatty acids; dodecanoid acid [ChEBI] fatty acids [KEGG: phytochemical compounds]
16	26.24	$C_{24}H_{34}O_6$	butanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester, [1aR-(1aa,2a,5ß,5aß,6ß,8aa,9a,10aa)]-	0.71	*cx*; *bx*	carboxylic acids; acyclic acids; butyric acid [MeSH] fatty acids [ChEBI] fatty acids [KEGG: phytochemical compounds]
17	26.59	$C_{32}H_{39}NO_{10}$	3-pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-oxo-4a,7a-epoxy-5H-cyclopenta[a]cyclopropa[f]cycloundecen-11-yl ester, [1aR-(1aR*,2R*,3S*,4aR*,6S*,7S*,7aS*,8E,10R*,11R*,11aS*)]-	0.07	*ox*	diterpenoids [CAS 51906-00-4]
18	28.01	$C_{22}H_{28}O_6$	quassin	2.48	*x*	terpenes [MeSH]
19	28.56	$C_{36}H_{69}NO_6Si_3$	methyl glycocholate, 3TMS derivative	0.13	*ox*	glycinated bile acids and derivatives [PubChem CID 22214169]

Table 6. Contd.

No.	RT (min)	Chemical formula	Phytochemical compounds	RA (%)	Bioactivities	Classifications
20	36.11	$C_{29}H_{48}O$	stigmasterol	3.26	*v*	alcohols; secondary alcohols; 3beta-hydroxy steroids [ChEBI] terpenoids; steroids [KEGG: phytochemical compounds]
21	38.62	$C_{16}H_{50}O_7Si_8$	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyloctasiloxane	0.09	*x*	siloxanes [CAS 19095-24-0]

Note: RT = retention time; RA = relative area; *ox* = antioxidant/radical scavenging activity; *bx* = antibacterial/antimicrobial; *cx* = cytotoxicity/anticancer/antiproliferative/anticarcinogenic; *x* = no activity reported with respect to *ox*, *bx*, and *cx*; *v* = *ox*, *bx*, and *cx* activities reported. See Supp. File 3.

Table 7. Identified phytochemical compounds of LM extract of *H. capitata*.

No.	RT (min)	Chemical formula	Phytochemical compounds	RA (%)	Bioactivities	Classifications
1	17.36	$C_{30}H_{48}O_2$	ergosta-5,22-dien-3-ol, acetate, (3ß,22E)- (brassicasterol)	0.28	*cx*; *ox*	terpenoids; cholestane steroids [LOTUS]
2	20.82	$C_{20}H_{38}$	neophytadiene	4.48	*ox*; *bx*	hydrocarbons; olefins; acyclic olefins [ChEBI] terpenoids; phytane diterpenoids [LOTUS]
3	21.70	$C_{17}H_{34}O_2$	methyl palmitate	8.28	*ox*; *bx*	fatty acids [MeSH]
4	22.36	$C_{18}H_{36}O_2$	ethyl palmitate	8.55	*ox*	fatty acids; palmitic acids [MeSH]
5	23.33	$C_{19}H_{34}O_2$	7,10-octadecadienoic acid, methyl ester	1.71	*x*	lineolic acids and derivatives [CAS 56554-24-6]
6	23.38	$C_{19}H_{36}O_2$	trans-13-octadecenoic acid, methyl ester	22.13	*cx*	fatty acid methyl esters [CAS 56554-47-3]
7	23.60	$C_{19}H_{38}O_2$	methyl isostearate	3.80	*x*	fatty esters; fatty acid methyl esters [CAS 5129-61-3] fatty acids [LOTUS]
8	23.85	$C_{26}H_{44}O_5$	ethyl iso-allocholate	0.60	*v*	terpenoids; cholane steroids [LOTUS]
9	24.00	$C_{20}H_{34}O_2$	ethyl linolenate	12.04	*x*	fatty acids [MeSH]
10	25.11	$C_{35}H_{68}O_5$	1,2-dipalmitoyl-sn-glycerol	2.68	*x*	fatty acids; diacylglycerols [LOTUS]
11	25.36	$C_{24}H_{46}O_2$	methyl 12-(2-octylcyclopropyl) dodecanoate	1.29	*ox*; *bx*	fatty acid esters; fatty acid methyl esters [HMDB0031018]
12	27.01	$C_{24}H_{36}O_6$	8,14-seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3ß-methoxy-4,4-dimethyl-	1.77	*cx*	ketals [PubChem CID 550132]
13	27.17	$C_{27}H_{40}O_4$	spirost-8-en-11-one, 3-hydroxy-, (3ß,5a,14ß,20ß,22ß,25R)-	0.43	*V*	11-oxosteroids [CAS 54965-96-7]
14	42.78	$C_{16}H_{50}O_7Si_8$	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyloctasiloxane	0.26	*X*	siloxanes [CAS 19095-24-0]

Note: RT = retention time; RA = relative area; *ox* = antioxidant/radical scavenging activity; *bx* = antibacterial/antimicrobial; *cx* = cytotoxicity/anticancer/antiproliferative/anticarcinogenic; *x* = no activity reported with respect to *ox*, *bx*, and *cx*; *v* = *ox*, *bx*, and *cx* activities reported. See Supp. File 3.

Discussion

Secondary metabolites of plants expressed a certain therapeutic potential and represent phytochemical compounds with bioactive properties. *H. capitata* contains phytochemical compounds that can be used to treat certain health problems. The phytochemical compounds identified in *H. capitata* extracts were alkaloids, saponins, flavonoids, tannins, and triterpenes/steroids (Table 1). However, the presence of all the phytochemical compounds was confirmed only in the RM extract. Alkaloids have antiproliferative, antibacterial, antioxidant potential which can be used to develop drugs (Dey et al. 2020). From a therapeutic point of view, alkaloids are particularly well-known as an anesthetic, cardioprotective, and anti-inflammatory agents (Heinrich et al. 2021). Saponins also possess a number of important pharmaceutical properties, such as anti-inflammatory, antifungal, antibacterial, antiparasitic, anticancer, and antiviral activities (Mugford & Osbourn 2013; Rai et al. 2021). Similar bioactivities have also been confirmed by *in vitro* assays that show tannins to have antiviral, antibacterial, enzyme inhibitory, antioxidant, radical scavenging, and antimutagenic properties (Serrano et al. 2009; Singh & Kumar 2019; Hilmi et al. 2021; Sabdoningrum et al. 2021).

Apart from alkaloids, saponins and tannins, flavonoids, triterpenes and plant sterols also have therapeutic properties. Flavonoids possess several beneficial qualities, such as anticarcinogenic, antioxidative, anti-inflammatory, and antiviral (Kumar & Pandey 2013; Panche et al. 2016; Sabdoningrum et al. 2021). Triterpenes also have promising antioxidant and antidiabetic activities and inhibit the formation of advanced glycation end products, which are involved in the pathogenesis of diabetic nephropathy, embryopathy, neuropathy, or impaired wound healing (Nazaruk & Borzym-Kluczyk 2015). In preclinical studies, triterpenes showed broad pharmacological effects including anticancer, antioxidant, anti-inflammatory, antiatherosclerotic, antiviral, hepatoprotective, and immunomodulatory activities (Renda et al. 2022). Plant steroids have many interesting medicinal, pharmaceutical, and agrochemical activities such as antitumor, anticancer, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, sex hormone, antihelminthic, cytotoxic, and cardiotonic activities (Salvador et al. 2013; Patel & Savjani 2015).

The antioxidant activity in Table 3 shows that RM has antioxidant activity categorised as potent. In the same way, antioxidant activity of the ethanol extract of *H. capitata*, which grows in Samarinda, is also classified as potent (Kusuma et al. 2020). The present study shows that the free radical scavenging activity is supported by the content of antioxidant -compounds, namely alkaloids, tannins, flavonoids and triterpenes (Panche et al. 2016; Chai et al. 2019; Maisetta et al. 2019; Li et al. 2020). Antioxidants act by preventing oxidation of a molecule. Alkaloids act as primary antioxidants by donating hydrogen atoms to free radicals (Kumaradewi et al. 2021). Similar to alkaloids, tannins also act as primary antioxidants. However, they also function as secondary antioxidants (Amarowicz 2006). Flavonoids as antioxidants can act as stimulants for internal antioxidant enzymes, counteract free radicals, inhibit free radical forming enzymes, and chelate metals (Prochazkova et al. 2011). Likewise, terpenoids act as radical scavengers (Luo et al. 2021). In addition to antioxidant activity, the anticancer potential of *H. capitata* extracts was also tested through *in vitro* cytotoxicity assay. Based on half inhibitory concentration (IC_{50}) of extracts in Table 4, it was known that RC, RM, and LM had moderate cytotoxic values. The phytochemical compounds of

each extract contributed to inhibiting the viability of cancer model cells, both Hela and 4T1. Saponins, flavonoids, triterpenoids and steroid compounds contained in the extracts were predicted to contribute to its cytotoxic activity. Saponins as well as triterpenoids, and flavonoids play role in triggering apoptosis, autophagy and are involved in cell cycle arrest (Bishayee et al. 2011; Kopustinskiene et al. 2020; Elekofehnti et al. 2021). Meanwhile, apart from increasing cellular antioxidant capacity, plant sterols are involved in inhibiting cell proliferation (Perens & Shahman 2005).

Analysis of phytochemical compounds by GC-MS on RC (Table 5), showed pregn-5-en-20-one, 3-hydroxy, (3ß,17a); methyl retinoate; ferruginol; quassin; stigmasterol, and campesterol had more RA from 1 %. Among phytochemical compounds, there was no reference on quassin bioactivity regarding antioxidant, and cytotoxicity. Meanwhile, the bioactivity of campesterol, such as antioxidant activity and cytotoxicity, has been confirmed by a number of records (Table 5). The remaining phytochemical compounds were confirmed for their bioactivities related to antioxidant, and cytotoxicity. The phytochemical compound of pregn-5-en-20-one, 3-hydroxy, (3ß,17a)- has already been identified by To'bungan et al. (2022b) in the chloroform extract of the root and its fraction of *H. capitata*, albeit in smaller amounts. The presence of ferruginol, stigmasterol, and campesterol is consistent with previous findings by To'bungan et al. (2022b).

Campesterol and stigmasterol, together with β-sitosterol, are the most abundant phytosterols, while ergosterols are less commonly found in plants. MTT assay of *Rhizophora apiculata* extract phytosterols against HeLa, MCF-7, and A549 demonstrate cytotoxicity and importance of aliphatic sterol moiety (Kurniawan et al. 2023). One of the three types of phytosterol isolated from *Rhizophora apiculata* have a moderate cytotoxic effect on HeLa, MCF-7, and A549 cells with IC_{50} values 71.20, 67.95, and 54.9µg/mL respectively (Kurniawan et al. 2023). The cytotoxicity of non-esterified stigmasterol has been reported to significantly affect human intestinal cell viability and proliferation through cell cycle arrest at the G_2/M phase and inhibition of DNA synthesis (Kasprzak et al. 2023). Stigmasterol also induces the apoptosis pathway by enhancement of intracellular reactive oxygen species (ROS) generation and caspase 3/7 activity of human intestinal cells (Kasprzak et al. 2023). Lee et al. (2021) obtained 14.58% ferruginol from GC-MS analysis of *Metasequoia glyptostroboides* with anticancer activities mediated by the presence of hydroxyl groups, including other biologically active components reported to be anticancer and/or antitumor against Hela cells. Terpene derivatives and terpenoids are the main classes of phytochemical compounds in RC extract. The phytochemical compounds found in RC extract were consistent with the findings in phytochemical screening. It also confirmed the cytotoxicity in HeLa and 4T1 cancer cells. However, it contradicted the finding in radical scavenging assay.

Phytochemical compound analysis by GCMS shows there were several compounds that were predicted to be contained in RM, namely gibberellin A44 (GA44), ferruginol; cis-13-octadecenoic acid methyl ester, methyl palmitate, stigmasterol, and quassin (Table 6). Among phytochemical compounds, there was no reference on quassin and gibberellin A44 (GA44) bioactivity in terms of antioxidant and cytotoxicity. Meanwhile, the bioactivities of methyl palmitate, as antioxidant activity by suppressing oxidative stress , have been confirmed by Hamed et al. (2020), and for cis-13-octadecenoic acid methyl ester, contained in *Curcuma longa*, is thought to be involved in its

anticancer properties (Anekwe et al. 2023). The remaining phytochemical compounds were confirmed for their bioactivities related to antioxidant, and cytotoxicity. The phytochemical compound ferruginol has already been identified by To'bungan et al. (2022b) in the chloroform extract of the root and its fraction of *H. capitata*, with a higher RA (%) in its fraction. To'bungan (2023) specifically reported the presence of ferruginol in the root methanolic extract of *H. capitata* and was thought to be involved in its cytotoxic activity against WiDr colon cancer cells. Terpene derivatives and terpenoids are the major classes of phytochemical compounds in RM extract. The phytochemical compounds found in RM extract are consistent with the findings in phytochemical screening and also radical scavenging assay. The radical scavenging activity of the RM extract was qualified as potent and could be highly correlated with the concentration of ferruginol, methyl palmitate, and stigmasterol. It also confirmed the cytotoxicity in *HeLa* and *4T1* cancer cells.

Trans-13-octadecenoic acid methyl ester, ethyl linolenate, ethyl palmitate, methyl palmitate, neophytadiene, and methyl isostearate are predicted to be contained in LM (Table 7). Among the phytochemical compounds, there was no reference on the bioactivity of ethyl linolenate and methyl isostearate in terms of antioxidant and cytotoxicity. Meanwhile, the bioactivities of methyl palmitate and neophytadiene, such as antioxidant activity have been confirmed by several records, and for ethyl palmitate, antioxidant activity has been reported. The bioactivities related to cytotoxicity were confirmed for the remaining phytochemical compounds of trans-13-octadecenoic acid methyl ester. Krishnamoorthy and Subramaniam (2014) classified trans-13-octadecenoic acid methyl ester as linoleic acid esters. Linoleic acid ester treatment reportedly enhances ER-mitochondrial contact formation (MERC) which in turn promoted calcium (Ca^{2+}) signaling, mitochondrial energetics, and $CD8^{+}$ T-cell (CTL) effector functions, thus improving antitumor activity (Lauson et al. 2023). The enhanced apoptotic effect of cisplatin in A549 cells when integrated with conjugated linoleic acid was also confirmed by Yuce et al. (2023).

The phytochemical compound trans-13-octadecenoic acid methyl ester has already been identified by To'bungan et al. (2022b) in the root chloroform extract of *H. capitata* with a lower RA (%). Fatty acid derivatives and fatty acid methyl esters were the major classes of phytochemical compounds in LM extract. The phytochemical compounds found in LM extract are consistent with the findings in phytochemical screening and also radical scavenging assay. The phytochemical compounds identified in the LM extract were also responsible for the cytotoxicity in the 4T1 cancer cells.

CONCLUSION

Both RC and RM showed moderate cytotoxicity to Both HeLa and 4T1 cells, whereas only LM showed moderate cytotoxicity to 4T1 cells. RM exhibited high antioxidant activity. Campesterol, ferruginol, stigmasterol, cis-13-octadecenoic acid methyl esters, and methyl palmitate are predicted to play a role in the cytotoxicity and antioxidant activity. RC, RM, and LM have the potential to be developed for anticancer purposes. In addition to being an anticancer, RM also has the potential to be developed as an antioxidant, based on its relatively radical scavenging activity. Further studies regarding the cytotoxic effects of RC, RM, LM on normal cells and their toxicity in experimental animals need to be investigated.

AUTHOR CONTRIBUTION

NT and SSW conceived, formulated, supervised, and designed the research. FNLH and IWSM performed the research and drafted the manuscript.

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CONFLICT OF INTEREST

There is no conflict of interest.

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