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Phytochemicals, essential oils, and bioactivities of an underutilized wild fruit Cili (*Rosa roxburghii*)

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ABSTRACT

In this study, the phytochemical and essential oil profiles, and bioactivities of an underutilized wild fruit Cili (*Rosa roxburghii*) were analyzed. Cili fruit was found to have higher antioxidant activities, ascorbic acid content (122 mg LAA/g DW), total phenolics (173 mg GAE/g DW), and total flavonoids (34.5 mg CE/g DW) than 11 selected common fruits and vegetables. Thirty phytochemical compounds were tentatively identified in the aqueous-ethanolic extract of Cili fruit using UPLC-QTOF-MS. Linoleic acid and 2, 2′-methylenebis (6-tert-butyl-4-methylphenol) were tentatively identified as the predominant essential oil components using GC–MS. The aqueous-ethanolic extract of Cili fruit was further tested for its *in vitro* bioactivities, and found it exhibited antibacterial activity against four multi-drug resistant strains of *Staphylococcus aureus*, and showed *in vitro* anti-proliferative effects against three cancer cells. Overall, the Cili fruit with diverse bioactive compounds and bioactivities can be applied in the food, nutraceutical, and cosmetic industries for the development of functional products.

1. Introduction

Regular consumption of fruits and vegetables has been demonstrated to be associated with a reduced risk of certain chronic diseases due to the presence of phytochemicals with diverse bioactivities (Kim et al., 2012; Zhu et al., 2019). L-ascorbic acid (LAA), the main biologically active form of vitamin C, is prominently found in many fruits and vegetables. Vitamin C has many health benefits, such as antioxidant, anticancer, and cardiovascular-protective effects (Ngo et al., 2019). Polyphenols are the most abundant phytochemicals in fruits and vegetables, and mainly include phenolic acids, flavonoids, proanthocyanidins, stilbenes, and lignans (Lorrain et al., 2013). It is widely reported that polyphenols exhibit multifold health benefits, e.g., antioxidant, anticancer, antidiabetic, antiaging, and neuroprotective effects (Yang et al., 2018). Moreover, volatile compounds in many fruits have also received increasing attention by researchers due to their healthy properties, such as cardiovascular-protective effect (Kim et al., 2016). Therefore, understanding of the phytochemical profile and bioactivities of fruits and vegetables can provide a scientific basis for their health benefits.

Cili (*Rosa roxburghii*) plant (Figure S1) is a perennial deciduous shrub in Rosaceae family (Liu et al., 2016). It is widely distributed in southwestern areas of China, such as Guizhou, Guangxi, Hunan, Sichuan, and Chongqing. Its fruits are edible and have been traditionally used as Chinese folk medicine for a long time (He et al., 2016). However, Cili fruit is an underutilized fruit as they are rarely available owing to their limited yield. Lack of scientific evidence on their nutritional values and health benefits has also intercepted the promotion and application of this wild fruit in the modern industries. In view of this, the present study was conducted to investigate the phytochemical and essential oil profiles, and antioxidant, antibacterial, and anti-proliferative activities of Cili fruit. The findings obtained will be useful for future utilization of Cili fruit in the food, nutraceutical, and cosmetic industries for the development of functional products.

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2. Materials and methods

2.1. Chemicals and reagents

Gallic acid, catechin, Folin–Ciocalteu reagent, L-ascorbic acid (LAA), 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2, 4, 6-tripyridyl-S-triazine (TPTZ), and 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) were purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade of methanol, ethanol, dimethyl sulfoxide (DMSO), and acetic acid were purchased from Taitan Scientific Co., Ltd. (Shanghai, China). Mueller Hinton (MH) broth was purchased from Oxiod (Basingstoke, England). Gentamicin was purchased from Meilune (Dalian, China). Dulbecco's minimal essential medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionized water was used in all the experiments.

2.2. Plant materials

Mature Cili fruits with overall the same maturity stage (Figure S1) was collected in the countryside of Tongliang county, Chongqing, China, in August, 2017. Its authenticity was confirmed by Prof. Hua-Bin Li from Department of Nutrition, School of Public Health, Sun Yat-Sen University. The voucher specimen was preserved in Department of Food Science & Technology, School of Agriculture and Biology, Shanghai Jiao Tong University. Eleven common fruits and vegetables, including blueberry, broccoli, chili, eggplant, green apple, kiwifruit, lemon, orange, spinach, strawberry, and tomato, were purchased from Auchan, a local supermarket in Shanghai, China. Cili fruit and other fruits and vegetables were freshly sliced, freeze-dried, milled into fine powders, and stored at 4 ℃ for further use.

2.3. Extraction of phytochemicals from mature Cili fruits

For the determination of ascorbic acid content, total phenolic content (TPC), total flavonoid content (TFC), antioxidant activities (FRAP, ABTS, and DPPH), and UPLC-QTOF-MS analysis of phytochemical compounds, freeze-dried sample powder (0.1 g) was mixed with 1.5 mL of ice-cold aqueous-ethanol (30:70, v/v) containing 0.1% formic acid, and the mixture was vigorously vortexed for 5 min at room temperature, followed by centrifugation for 10 min (12,000 × *g*, 4 ℃), and the supernatant was collected and stored at 4 ℃ for further analysis within a week.

For GC–MS analysis of the essential oil profile of Cili fruit, the extraction was performed based on the method of a previous study (Liu et al., 2016) with some modifications. Briefly, freeze-dried sample powder (0.1 g) was extracted with 2 mL of *n*-hexane for 6 h, followed by centrifugation for 15 min (12,000 × *g*, 4 ℃). The supernatant was collected and dehydrated with excess anhydrous sodium sulfate, and this sample was regarded as the *n*-hexane extract. The pellet after centrifugation was further extracted with 2 mL of ethyl acetate for 6 h, and then the mixture was centrifuged for 15 min (12,000 × *g*, 4 ℃). The supernatant was collected and dehydrated with excessive anhydrous sodium sulfate, and this sample was regarded as the ethyl acetate extract. Both extracts were stored at 4 ℃ until use.

For estimating the antibacterial and antiproliferative activities of Cili fruit, the aqueous-ethanolic extract was further freeze-dried and dissolved in deionized water to make a stock solution with the final concentration of 25 mg/mL.

2.4. Determination of TPC

The Folin-Ciocalteu method was used to determine the TPC of aqueous-ethanolic extracts according to Gan et al. (2010). Briefly, properly diluted sample solution (0.4 mL), Folin-Ciocalteu reagent (2.0 mL), and Na_2CO_3 solution (75 g/L, 1.6 mL) were mixed well and reacted in dark at room temperature for 2 h and recorded its absorbance at 760 nm. Gallic acid was used as a standard (0-0.1 mg/mL), and the results were expressed as milligrams of gallic acid equivalent per gram dry weight (mg GAE/g DW) of Cili fruit.

2.5. Determination of TFC

The TFC of aqueous-ethanolic extracts were determined by $AlCl_3$-based colorimetric method as previously described (Gan et al., 2016). Briefly, deionized water (3.5 mL) was added to properly diluted sample solution (0.5 mL) and mixed well. $NaNO_2$ solution (0.5 M, 0.15 mL) was added to the mixture, mixed well, and reacted for 6 min. After that, $AlCl_3$ solution (0.3 M, 0.15 mL) was added to the mixture, mixed well, and further reacted 5 min. Finally, NaOH solution (1 M, 1 mL) was added to the mixture, mixed well, and the absorbance was recorded at 510 nm. Catechin was used as a standard (0-0.5 mg/mL), and the results were expressed as milligrams of catechin equivalent per gram dry weight (mg CE/g DW) of Cili fruit.

2.6. Determination of antioxidant activities

The antioxidant activities of aqueous-ethanolic extracts were evaluated with ferric reducing antioxidant power (FRAP) assay, DPPH radical scavenging activity (DPPH) assay, and 2, 2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity (ABTS) assay based on the methods described in previous studies (Gan et al., 2010; Yang et al., 2019), and the results were expressed as μmol Fe (II)/g DW, μmol Trolox/g DW, and μmol Trolox/g DW of Cili fruit, respectively.

2.7. Determination of ascorbic acid content by HPLC

Ascorbic acid content of Cili fruit was determined using a HPLC system (Shimadzu, Kyoto, Japan) coupled with a diode-array detector (DAD). A C_{18} column (4.6 mm × 250 mm, 5 μm) connected with a guard column (Shimadzu, Kyoto, Japan) was used to separate the samples. The mobile phases consisted of 5 mmol/L KH_2PO_4, pH 2.65 (solvent A) and methanol (solvent B). Separation was done based on the following program: 0 min, 5% B; 6 min, 22% B, and then returned to the initial condition within the next 9 min. The injection volume was 20 μL and the flow rate was set at 0.8 mL/min. Ascorbic acid in the samples were detected at 245 nm. Quantification of ascorbic acid was carried out according to the calibration curve using L-ascorbic acid (LAA) as a standard (0.01-0.1 mg/mL). The results were expressed as milligrams of LAA equivalent per gram dry weight (mg LAA/g DW) of Cili fruit.

2.8. Phytochemical profile analysis by UPLC-QTOF-MS

Qualitative analysis of phytochemical compounds in Cili fruit aqueous-ethanolic extract was performed using a UPLC system equipped with quadrupole TOF mass spectrometer (Waters, Milford, MA). The chromatographic separation was performed on a BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm) with the following settings: column temperature, 35 °C; flow rate, 0.4 mL/min; mobile phase A, deionized water containing 0.1% (v/v) formic acid; mobile phase B, acetonitrile; injection volume, 2 μL. The gradient condition was: 0 min, 2% B; 10 min, 60% B; 12 min, 60% B; 13 min, 98% B; 13.1 min, 2% B; 15 min, 2% B. The quadrupole TOF mass spectrometer was used for its ability to acquire MS/MS spectra on an information-dependent basis (IDA) during an LC/MS experiment. In this mode, the acquisition software (Analyst TF 1.7, AB Sciex) continuously evaluated the full scan survey MS data as it collected and triggered the acquisition of MS/MS spectra depending on preselected criteria. In each cycle, 12 precursor ions with intensity greater than 100 were chosen for fragmentation at collision energy (CE) of 30 V (15 MS/MS events with each product ion

accumulation time of 50 msec each). Acquisition of mass range was divided into 50–300, 290–600, 590–900, and 890–1500 with 4 injections. ESI source conditions were set as follows: ion source gas 60 Psi, curtain gas 35 Psi, source temperature 650 ℃, ion spray voltage floating (ISVF) 5000 V or −4000 V in positive or negative modes, respectively.

2.9. Essential oil profile analysis by GC–MS

A 7890/5975 GC/MSD instrument (Agilent, Santa Clara, CA, USA) was used to analyze the essential oil profile of Cili fruit. An Agilent HP-5MS capillary column (30 m × 250 mm ×0.25 μm) was applied to separate the essential oil components in the sample extracts. The oven temperature was initially set at 50 ˚C for 1 min, and then increased to 160 ˚C at a rate of 15 ˚C/min, and then increased again by 5 ˚C/min to a final temperature of 280 ˚C. The sample extract (5 μL) was injected into the GC system by a splitless injection. Helium was used as the carrier gas and the flow rate was set at 1.0 mL/min. The MS was operated in full scan mode with ionization energy of 70 eV. The relative contents of the detected compounds were quantified based on the area of each peak and the total areas of all peaks.

2.10. Determination of antibacterial activity

2.10.1. Detection of the diameter of inhibition zone (DIZ)

Four food-borne multi-drug resistant strains of *Staphylococcus aureus* (SJTU20857, SJTUF20745, SJTUF20758, and SJTUF20978) and a drug-sensitive *S. aureus* ATCC 25923, kind gifts from Prof. Chunlei Shi (Shanghai Jiao Tong University), were used to estimate the antibacterial activity of Cili fruit aqueous-ethanolic extract by agar well diffusion assay according to a previously reported study with some modifications (Shan et al., 2007). Briefly, a single colony of bacteria picked from MH plate was inoculated in MH broth overnight. The bacterial suspension (100 μL) diluted to about 1×10^6 CFU/mL with sterile LB medium was evenly spread onto the surface of MH agar plates by sterile glass beads (6 mm in diameter), and then 60 μL of Cili fruit extract (25 mg/mL) were added into Oxford cups (sterilized hollow cylinder with an inner diameter of 6 mm, outer diameter of 7.8 mm, and height of 10 mm), which were placed lightly on the agar surface in advance. Gentamicin (30 μg/mL) was used as the positive control, while deionized water (60 μL/cup) was used as the negative control. The plates were afterward incubated at 37 ˚C for 24 h for bacterial growth in an incubator (BI-150A, Shanghai Stik Co., Ltd, Shanghai, China). After the incubation period, diameters of the inhibitory zones (DIZs) formed surrounding the Oxford cups were measured to evaluate the antibacterial activity and expressed in millimeter (mm). The sample with DIZ value less than 8 mm was considered as “no inhibition zone (NIZ)”. All experiments were performed in triplicate.

2.10.2. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration for Cili fruit aqueous-ethanolic extract was evaluated by a broth microdilution method (Chan et al., 2018). The five different strains of *S. aureus* cultured overnight at 37 ˚C were diluted to get a final concentration of 10^6 CFU/mL. The various concentrations of Cili fruit extract (0.78–25 mg/mL) and gentamicin (0.78–50 μg/mL) were prepared by double dilution method in 96-well plate and 100 μL of bacterial suspension was added into each well to get the final concentration of 5×10^5 CFU/mL. The plates were incubated at 37 ˚C for 24 h. The MIC value was defined as the lowest concentration of extract that does not induce any visible turbidity in the well.

2.11. Anti-proliferative assay

MDA-MB-468 and MCF-7 human breast cancer cells, kind gifts from Prof. Jicun Ren (Shanghai Jiao Tong University) and HCT116 human colon cancer cells, a kind gift from Dr. Yueliang Zhao (Shanghai Ocean University), were used in this study. All cell lines were maintained in DMEM medium supplemented with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 ˚C in a humidified atmosphere with 5% CO_2. The anti-proliferative activity of Cili fruit aqueous-ethanolic extract on cancer cells was measured using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. Briefly, cancer cells were seeded into 96-well plates (5×10^3 cells/well) and incubated overnight at 37 ˚C in a humidified atmosphere with 5% CO_2. Cili fruit extract was diluted into various concentrations (0.15625–2.5 mg/mL) with the medium and added into 96 well plates, and incubated for 24 h. After that, 20 μL of MTT (5 mg/mL) was added and incubated for 4 h. The MTT solution was replaced with 150 μL DMSO and incubated for another 30 min. The absorbance was measured at 490 nm and the IC_{50} values were calculated.

2.12. Statistical analysis

All the experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). The data were analyzed with one-way ANOVA plus *post-hoc* Tukey test using statistical software IBM SPSS Statistics 19 (IBM, New York, NY, USA) to compare the mean values, with $p < 0.05$ defined as statistical significance.

3. Results and discussion

3.1. Contents of ascorbic acid, total phenolics, and total flavonoids in Cili fruit

The content of ascorbic acid is a prior factor for evaluating the quality of many fruits and vegetables (Ma et al., 2019). Our study found that ascorbic acid was one of the iconic nutrients in Cili fruit, which contained a much higher content (122 mg LAA/g DW) than the selected common fruits and vegetables (0.0–7.15 mg LAA/g DW), such as kiwifruit, strawberry, blueberry, lemon, orange, tomato, broccoli, spinach, chili, and green apple (Table 1). In addition, the ascorbic acid content of Cili fruit was higher than the published value (98.35 mg/g DW) (Zeng et al., 2017), which might be explained by different growing environments and extraction conditions. Overall, Cili fruit was rich in ascorbic acid, and could be a good natural source of dietary vitamin C.

Previous studies reported that many fruits and vegetables were also rich in other compounds, especially polyphenols (Du et al., 2009; Ma et al., 2019). In this study, we also evaluated the TPC and TFC of Cili fruit, and compared them with those selected fruits and vegetables (Table 1). Similar to the results of ascorbic acid, Cili fruit was found to contain much higher TPC (173 mg GAE/g DW) than the selected fruits and vegetables, such as strawberry (25.9 mg GAE/g DW), blueberry (18.9 mg GAE/g DW), and lemon (14.9 mg GAE/g DW). The TPC of Cili fruit in this study was a little higher than its previously reported values (109–156 mg GAE/g DW) (He et al., 2016; Zeng et al., 2017). For TFC, Cili fruit again had the highest value (34.5 mg CE/g DW) compared to the selected fruits and vegetables (0.42–9.47 mg CE/g DW) (Table 1). Previous studies reported the TFC of Cili fruit ranged from 47 to 114 mg rutin equivalent/g DW (He et al., 2016), equal to 22.3–54.2 mg CE/g DW, generally in agreement with our results.

However, previous studies indicated that in plant-based extracts, the presence of reducing compounds other than polyphenols, especially ascorbic acid, could contribute to an overestimation of TPC values due to its interference with the Folin-Ciocalteu assay (Everette et al., 2010; Sánchez-Rangel et al., 2013). As it was found that Cili fruit was rich in ascorbic acid, it was speculated that TPC in this study based on the Folin-Ciocalteu assay could be significantly overestimated. Therefore, the contribution of ascorbic acid to the TPC of Cili fruit was further estimated. The results showed that ascorbic acid contributed to 44% of the TPC in Cili fruit which was in agreement with our hypothesis. Besides Cili fruit, ascorbic acid, more or less, also contributed to the TPC of selected common fruits and vegetables. For example, it was found

Table 1
The ascorbic acid content (AAC), total phenolic content (TPC), and total flavonoid content (TFC) in Cili fruit and selected common fruits and vegetables.

Sample name	AAC (mg LAA/g DW)	TPC (mg GAE/g DW)		TFC (mg CE/g DW)
		Total	LAA-related	
Cili (*Rosa roxburghii*)	122 ± 5.20^{a}	173 ± 8.80^{a}	76.9 ± 4.92^{a}	34.5 ± 1.50^{a}
Broccoli (*Brassica oleracea* L.var.italic)	0.63 ± 0.04^{c}	8.57 ± 0.05^{de}	0.59 ± 0.04^{c}	2.82 ± 0.07^{ef}
Chilli (*Capsicum annuum* L.)	7.15 ± 0.16^{b}	14.6 ± 0.19^{cd}	5.88 ± 0.13^{b}	0.77 ± 0.04^{g}
Eggplant (*Solanum melongena* L.)	0.18 ± 0.16^{c}	6.54 ± 0.07^{e}	0.19 ± 0.05^{c}	3.20 ± 0.02^{de}
Spinach (*Spinacia oleracea* L.)	0.33 ± 0.28^{c}	14.7 ± 0.28^{cd}	0.32 ± 0.01^{c}	3.93 ± 0.15^{de}
Tomato (*Lycopersicon esculentum* Mill.)	5.53 ± 0.19^{b}	7.71 ± 0.29^{de}	4.57 ± 0.15^{bc}	1.54 ± 0.04^{fg}
Kiwifruit (*Actinidia Chinensis*)	3.84 ± 0.08^{b}	4.47 ± 0.05^{e}	3.19 ± 0.07^{bc}	0.42 ± 0.04^{g}
Strawberry (*Fragaria* × *ananassa* Duch.)	5.85 ± 0.23^{b}	25.9 ± 0.40^{c}	4.83 ± 0.18^{bc}	6.80 ± 0.24^{c}
Blueberry (*Vaccinium Spp*)	0.12 ± 0.05^{c}	18.9 ± 0.69^{b}	0.17 ± 0.04^{c}	9.47 ± 0.20^{b}
Lemon (*Citrus limon* L Burm.)	3.13 ± 0.10^{b}	14.9 ± 0.60^{cd}	2.61 ± 0.08^{bc}	7.18 ± 0.15^{c}
Orange (*Citrus sinensis*)	3.83 ± 0.16^{b}	9.16 ± 0.35^{de}	3.18 ± 0.05^{bc}	0.57 ± 0.02^{g}
Green apple (*Malus domestica*)	0.00^{c}	7.57 ± 0.27^{de}	0.00^{c}	4.31 ± 0.10^{d}

Each measurement was determined in triplicate, and the results were expressed as mean ± SD. One-way analysis of variance (ANOVA) plus *post hoc* Tukey test was performed to compare means. Different superscript lowercase number indicated statistical significance ($p < 0.05$).

that 72%, 59%, and 40% of the TPC values were related to ascorbic acid in kiwifruit, tomato, and chili, respectively. These results suggest that TPC of fruits and vegetables might be overestimated in some previous studies if the influence of ascorbic acid was not carefully considered.

3.2. Antioxidant activity of Cili fruit

In this study, different *in vitro* antioxidant assays, including the FRAP, ABTS, and DPPH assays with different antioxidant mechanisms (Shukla et al., 2016), were applied to evaluate the antioxidant activity of Cili fruit extract, which was compared with the selected common fruits and vegetables, with the results shown in Table 2.

The reducing power of samples was evaluated using FRAP assay, and Cili fruit extract exhibited a strong reducing power (2516 μmol Fe (II)/g DW), which was significantly higher than that of tested common fruits and vegetables (45.4–231 μmol Fe(II)/g DW) (Table 2). Similarly, Cili fruit extract also showed the strongest ABTS scavenging ability (1081 μmol Trolox/g DW), compared to other tested common fruits and vegetables (16.0–133 μmol Trolox/g DW). In addition, Cili fruit extract could significantly quench DPPH free radical with a value of 1140 μmol Trolox/g DW, which was significantly higher than that of tested common fruits and vegetables (18.9–172 μmol Trolox/g DW). Considering the high antioxidant activities of Cili fruit, we further investigated its specific bioactive compounds by UPLC-QTOF-MS, and evaluated its antibacterial and anti-proliferative effects. GC–MS analysis was also conducted to identify the volatile compounds in the Cili fruit.

3.3. Characterization of phytochemicals of Cili fruit

Cili fruit also contained abundant phytochemicals in its aqueous-ethanolic extract, which were analyzed by UPLC-QTOF-MS, with the spectrum of Cili fruit ethanol extract shown in Figure S2A. Three organic acids were tentatively identified, including quinic acid, ascorbic acid, and ceanothic acid (Table 3). Quinic acid (compound 1) and ascorbic acid isomers (compound 2 and 3) were previously identified in Cili fruit (Liu et al., 2016). However, this study is the first to report the presence of ceanothic acid isomers (compound 27 and 30) in this fruit.

Five flavonoids and three procyanidins were tentatively identified in Cili fruit (Table 5). Gallocatechin (compound 5) and catechin (compound 8) were tentatively identified according to the literature data (Dai et al., 2015). Both compounds 13 and 14 were tentatively identified as hydroxykaempferol-O-hexose isomers with [M − H]– at *m/z* 463.0877, and an MS^2 product ion at *m/z* 301, the latter reflecting the loss of hexosyl [M − H – 162]–. Compound 16 was tentatively identified as quercetin-O-(O-acetyl)-hexose with deprotonated molecular ion at *m/z* 505.0989, because it showed the loss of (O-acetyl)-hexosyl and gave an MS^2 signal at *m/z* 301.02648, which matched with the molecular weight of quercetin (Liu et al., 2016), and the fragment ion at *m/z* 463.08726 was obtained by losing acetyl moiety. Compound 18 was tentatively identified as hydroxyl-dimethoxyflavone-O-hexose with a molecular ion [M + HCOO]– at *m/z* 521.1309, which lost one methoxy moiety and hexosyl (*m/z* 161.0449), giving a product ion at *m/z* 280.03947. The fragment ions at *m/z* 249.11264 and 183.02904 were obtained by the cleavage of bond 1 and 3, respectively. Compounds 6,

Table 2
Antioxidant activities of Cili fruit and selected common fruits and vegetables.

Sample name	FRAP (μmol Fe(II)/g DW)		ABTS (μmol Trolox/g DW)		DPPH (μmol Trolox/g DW)	
	Total	LAA-related	Total	LAA-related	Total	LAA-related
Cili (*Rosa roxburghii*)	2516 ± 118^{a}	1416 ± 94.1^{a}	1081 ± 30.1^{a}	623 ± 37.2^{a}	1140 ± 118^{a}	665 ± 38.3^{a}
Broccoli (*Brassica oleracea* L.var.italic)	47.2 ± 0.54^{e}	7.49 ± 0.60^{c}	16.0 ± 0.16^{e}	4.55 ± 0.24^{c}	18.9 ± 0.50^{d}	5.27 ± 0.24^{c}
Chilli (*Capsicum annuum* L.)	152 ± 1.36^{bcd}	95.4 ± 2.17^{b}	63.9 ± 0.92^{cd}	39.3 ± 0.86^{b}	56.4 ± 3.00^{cd}	41.0 ± 0.88^{b}
Eggplant (*Solanum melongena* L.)	45.4 ± 0.52^{e}	2.51 ± 0.85^{c}	19.0 ± 0.26^{e}	2.58 ± 0.33^{c}	21.7 ± 0.50^{d}	3.24 ± 0.34^{c}
Spinach (*Spinacia oleracea* L.)	69.7 ± 1.36^{de}	5.57 ± 0.26^{c}	28.4 ± 0.70^{e}	3.79 ± 0.10^{c}	26.7 ± 1.70^{cd}	4.49 ± 0.11^{c}
Tomato (*Lycopersicon esculentum* Mill.)	74.0 ± 3.22^{de}	73.6 ± 2.56^{bc}	31.0 ± 0.93^{e}	30.7 ± 0.99^{bc}	36.4 ± 1.87^{cd}	32.2 ± 1.02^{bc}
Kiwifruit (*Actinidia Chinensis*)	55.1 ± 0.95^{de}	50.8 ± 1.08^{bc}	23.4 ± 0.47^{e}	21.7 ± 0.43^{bc}	26.3 ± 0.78^{cd}	22.9 ± 0.44^{bc}
Strawberry (*Fragaria* × *ananassa* Duch.)	231 ± 10.8^{b}	77.9 ± 3.07^{bc}	133 ± 2.38^{b}	32.4 ± 1.21^{bc}	172 ± 3.49^{b}	33.9 ± 1.25^{bc}
Blueberry (*Vaccinium Spp*)	195 ± 6.51^{bc}	0.53 ± 0.68^{c}	89.3 ± 2.86^{c}	1.80 ± 0.27^{c}	123 ± 2.65^{bc}	2.44 ± 0.28^{c}
Lemon (*Citrus limon* L Burm.)	112 ± 5.13^{cde}	41.1 ± 1.37^{bc}	39.2 ± 2.15^{de}	17.9 ± 0.54^{c}	55.9 ± 1.93^{cd}	19.0 ± 0.56^{bc}
Orange (*Citrus sinensis*)	76.4 ± 2.12^{de}	50.6 ± 0.80^{bc}	23.7 ± 0.59^{e}	21.6 ± 0.32^{bc}	30.5 ± 0.99^{cd}	22.8 ± 0.33^{bc}
Green apple (*Malus domestica*)	58.7 ± 2.18^{de}	0.00^{c}	33.9 ± 0.73^{e}	0.00^{c}	47.8 ± 1.20^{cd}	0.00^{c}

Each measurement was determined in triplicate, and the results were expressed as mean ± SD. One-way analysis of variance (ANOVA) plus *post hoc* Tukey test was performed to compare means. Different superscript lowercase number indicated statistical significance ($p < 0.05$).

Table 3
Phytochemical profile of Cili fruit based on UPLC-QTOF-MS/MS.

No.	Compounds	Rt (min)	Formula	Mass error (Da)	MS^1 (m/z)	MS^2(m/z)	Adducts
1	Quinic acid	0.67	$C_7H_{12}O_6$	−0.5	191.0556	173.04482, 127.03944	-H
2	Ascorbic acid	0.74	$C_6H_8O_6$	−0.6	175.0242	115.00318. 87.00822	-H
3	Ascorbic acid isomer	0.80	$C_6H_8O_6$	−0.5	175.0243		-H
4	Galloyl-hexose	1.45	$C_{13}H_{16}O_{10}$	−0.6	331.0665	169.01348, 125.02367	-H
5	Gallocatechin	3.28	$C_{15}H_{12}O_7$	−1.2	305.0661	167.03421, 139.03955, 125.02368, 109.02870	-H
6	Procyanidin dimer A type	3.54	$C_{30}H_{24}O_{12}$	0.2	575.1197	465.08005, 359.07636, 289.07064, 167.03414, 125.02370, 109.02892	-H
7	Procyanidin dimer B type	3.74	$C_{30}H_{26}O_{12}$	−0.1	577.135	451.10230, 109.02879, 149.02355, 425.08659, 407.07607, 289.07074, 271.05946, 179.03400,	-H
8	Catechin	3.83	$C_{15}H_{14}O_6$	−0.4	289.0713	271.06120, 179.03413, 109.02879, 167.03404, 163.03912, 151.03918, 137.02368, 121.02883	-H
9	Procyanidin dimer B type	4.10	$C_{30}H_{26}O_{12}$	−0.1	577.135	109.02891, 151.03924, 425.08686, 451.10252, 289.07087, 179.03405	-H
10	1-O-Galloylpedunculagin	4.20	$C_{41}H_{28}O_{26}$	1	935.0806	765.05794, 633.07264, 481.06192, 463.05098, 331.06807, 300.99815, 291.01401, 275.01890, 259.02295, 249.03947	-H
11	Tamariscinoside B	4.55	$C_{26}H_{36}O_{12}$	1.1	539.2145	173.02302, 160.01569, 144.02098, 132.02096	-H
12	Ellagic acid	4.69	$C_{14}H_6O_8$	−0.4	300.9986	283.98843, 257.00834	-H
13	Hydroxykaempferol-O-hexose	4.90	$C_{21}H_{20}O_{12}$	−0.5	463.0877	301.02645, 177.03956	-H
14	Hydroxykaempferol-O-hexose	4.99	$C_{21}H_{20}O_{12}$	−0.4	463.0878	301.02755	-H
15	Tamariscinoside B	5.14	$C_{27}H_{37}O_{14}$	−0.2	585.2187	33013143, 315.08623, 303.12239, 273.11250, 168.07844, 124.05226	+HCOO
16	Quercetin-O-(O-acetyl)- hexose	5.29	$C_{23}H_{22}O_{13}$	0.2	505.0989	463.08726, 301.03538	-H
17	Pinoresinol-O-hexose	5.79	$C_{26}H_{32}O_{11}$	−0.3	519.1869	357.13344, 342.11001	-H
18	Hydroxy-dimethoxyflavone-O-hexose	6.91	$C_{23}H_{24}O_{11}$	0.9	521.1309	280.03974, 249.11264, 183.02904, 161.04494	+HCOO
19	Chebuloside II	8.02	$C_{36}H_{58}O_{11}$	0.2	711.3963	503.33641, 485.32506, 457.32966, 441.33536	+HCOO
20	Picfeltarraenin X	8.28	$C_{36}H_{56}O_{11}$	0.1	709.3805	501.32058	+HCOO
21	Chebuloside II	9.17	$C_{36}H_{58}O_{11}$	0.3	711.3965	503.33666, 485.32590, 441.33640	+HCOO
22	Huangqiyenin A	9.95	$C_{36}H_{58}O_{10}$	0.6	695.4018	487.34198, 441.33639, 425.34146	+HCOO
23	Huangqiyenin A	10.19	$C_{36}H_{58}O_{10}$	0.3	695.4015	592.36271, 487.34181	+HCOO
24	Huangqiyenin A	10.42	$C_{36}H_{58}O_{10}$	0.3	695.4015	487.34169, 467.31566, 441.33616, 437.30528, 425.34107	+HCOO
25	Periplocoside O	10.58	$C_{36}H_{56}O_{10}$	0.2	693.3858	503.33665, 485.23609, 481.22013	+HCOO
26	16-Oxo alisol A	11.75	$C_{30}H_{48}O_6$	−0.4	503.3374	485.32603, 473.32481, 455.31568, 439.32042	-H
27	Ceanothic acid	12.04	$C_{30}H_{46}O_5$	−0.4	485.3268	467.31543, 425.30512, 393.31513	-H
28	Melianodiol	12.25	$C_{30}H_{48}O_5$	−0.4	487.3425	469.33102, 451.32117	-H
29	Melianodiol	12.30	$C_{30}H_{48}O_5$	−0.3	487.3426	451.32177,437.30483	-H
30	Ceanothic acid	12.96	$C_{30}H_{46}O_5$	−0.5	485.3268	467.31543, 441.33613, 437.30480, 425.30503, 411.32543, 407.29427	-H

7, and 9 were tentatively identified as an A-type procyanidin dimer, a B-type procyanidin dimer, and a B-type procyanidin dimer, respectively, according to the published data (Liu et al., 2016; Rahman et al., 2018). Compound 12 was tentatively identified as ellagic acid with [M−H]– at *m/z* 300.9986 and fragment ions at *m/z* 283.98843 and 257.00834, in agreement with a previous study (Alves de Souza and de, 2018).

Galloyl-hexose (compound 4) was tentatively identified due to its deprotonated molecular ion at *m/z* 331.0665, followed by the loss of hexosyl group [M−H – 162]–, thus giving an ion signal at *m/z* 169.01348, which matched with the literature data (Taamalli et al., 2014). Compound 10 was tentatively identified as 1-O-galloylpedunculagin according to its [M−H]–at *m/z* 935.0806 and fragment ions at *m/z* 765.05794 [M−H – gallic acid]–, 633.07264 [M−H – galloy dimer]–, 481.06192 [M−H – galloy dimer – galloy]–, 463.05098 [M−H – galloy dimer – gallic acid]–, 331.06807 [M−H – galloy dimer – galloy dimer]–, 300.99815 [galloy dimer]–, 291.01401 [gallic acid dimer – CO_2]–, 275.01890 [galloy dimer – CO]–, 259.02295 [M – gallic acid dimer – gallic acid dimer]–, and 249.03947 [gallic acid dimer – CO_2 – CO_2]–. Pinoresinol-O-hexose (compound 17) was tentatively identified with its deprotonated molecular ion at *m/z* 519.1869, which lost a hexosyl moiety [M−H – 162]– and gave a product ion at *m/z* 357.13344 matching with the molecular weight of pinoresinol (Liu et al., 2016). The latter further lost a methyl moiety giving a product ion at *m/z* 342.11001. According to the previous literature data (Mncwangi et al., 2014), compound 19 and 21 were both identified as chebuloside II isomers with their molecular ion [M + HCOO]– at *m/z* 711 and similar fragment ions. Picfeltarraenin X (compound 20) was tentatively identified with its molecular ion [M + HCOO]– at *m/z* 709.3805, which lost a hexosyl moiety giving a fragment ion at *m/z* 501.32058. Compound 22, 23, and 24 had the same molecular ion [M + HCOO]– at *m/z* 695 and were all tentatively identified as huangqiyenin A isomers. The fragment ion at *m/z* 487 was obtained by losing a hexosyl group [M−H – 162]–, which further lost a molecular water and a methyl moiety giving a fragment ion at *m/z* 441. Compounds 25, 28, and 29 were all triterpenoids and tentatively identified according to the mass data provided by Mass software (Analyst TF 1.7, AB Sciex).

3.4. Characterization of the essential oil profile in Cili fruit

The essential oil is another important group of bioactive constituents of Cili fruit. In this study, the *n*-hexane and ethyl acetate extracts of Cili fruit were analyzed using GC–MS and the total ion chromatograms were shown in Figure S2B and Figure S2C, respectively. As shown in Table 4, 59 compounds were tentatively identified in the *n*-hexane extract and ethyl acetate extract, but the compositions of both extracts were different. In its *n*-hexane extract, 32 compounds were found, representing 72.99% of the *n*-hexane extract. It was amazing that 30.57% of the *n*-hexane extract was linoleic acid, a polyunsaturated fatty acid possessing manifold health effects, such as anti-obesity (den Hartigh, 2019), anti-inflammatory (Ma et al., 2019), reducing anxiety, and preventing lipid peroxidation (Queiroz et al., 2019). Interestingly, Cili fruit even contained a higher percentage of linoleic acid than some well-known linoleic acid-rich seeds, such as pot marigold (29.5%), mahaleb (27.6%), and catappa (27.5%) (Özgül-Yücel, 2005). In addition, its *n*-hexane extract also contained a relatively high amount of 2, 2′-methylenebis (6-tert-butyl-4-methylphenol) (13.37%), which is a popular antioxidant (Tan et al., 2017) and anti-aging agent (Yamada, 2018). Besides, it can also enhance the antitumor activity of chemotherapy drugs (Jang et al., 2017). On the other hand, in its ethyl acetate extract, 27 compounds were detected, representing 76.92% of the ethyl acetate extract, among which 2, 2′-methylenebis

Table 4
Essential oil profile of Cili fruit based on GC–MS/MS.

No.	Rt (min)	Compounds	Main Mass Fragments	Area%	Ref.	CAS	Qual.[a]
n-Hexane extract							
1	7.362	2,2,4,6,6-pentamethyl- Heptane	155.0, 113.0, 99.0, 85.0, 71.0, 57.0	0.10	38358	013475-82-6	59
2	8.877	1-Methyl-2-pyrrolidinone	99.0, 84.9, 71.0, 44.0	0.03	3503	000872-50-4	86
3	9.238	4-Methoxy-2,5-dimethyl-3(2 H)-furanone	142.0, 99.0, 71.0, 43.0	0.10	19506	004077-47-8	60
4	9.743	2-Acetylcyclopentanone	125.9, 111.1, 57.0, 43.0	0.04	11155	001670-46-8	64
5	10.392	4-Methyl-undecane	170.1, 85.0, 71.0, 57.0, 43.0	0.12	38326	002980-69-0	62
6	11.114	(E)-1-Methyl-1-propenylbenzene	131.9, 117.0, 88.9	0.01	14096	000768-00-3	86
7	11.547	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	143.9, 101.0, 72.0, 43.0	0.02	20638	028564-83-2	87
8	12.413	Octanoic acid	115.0, 101.0, 85.0, 73.0, 59.9, 43.0, 29.9	0.06	20837	000124-07-	94
9	13.567	Ethyl nicotinate	151.0, 123.0, 106.0, 78.0, 50.9	0.06	24804	000124-07-	94
10	14.577	1,4-Bis(1,1-dimethylethyl)-benzene	190.1, 175.1, 117.0, 105.0, 91.0, 57.0, 41.0	0.05	53170	001012-72-2	63
11	15.299	2-Methyldecane	141.0, 99.0, 85.0, 71.0, 57.0, 43.0	0.09	28427	006975-98-0	64
12	15.876	2,3,7-trimethyldecane	97.0, 71.0, 43.0	0.07	48887	062238-13-5	63
13	17.247	2-(1,1-Dimethylethyl)-4-methylphenol	165.0, 149.0, 121.0, 91.0, 41.0	0.18	33649	002409-55-4	93
14	17.608	n-Decanoic acid	172.0, 129.0, 73.0	0.08	39474	000334-48-5	98
15	18.618	(+)-Valencene	204.0, 161.0, 142.0, 133.0, 105.0, 91.0, 79.0, 41.0	0.14	64543	004630-07-3	98
16	18.979	1,3-Benzenediacetonitrile	155.9, 131.0, 116.0, 101.0, 89.0	0.07	28413	000626-22-2	69
17	20.422	D-Allose	163.0, 144.0, 115.0, 98.0, 60.0, 43.0	0.41	45881	002595-97-3	64
18	21.216	Butylatedhydroxytoluene	205.0, 177.0, 145.0, 105.0, 91.0, 81.0, 57.0	0.78	77555	000128-37-0	96
19	21.504	l-Calamenene	202.0, 159.0, 144.0, 129.0, 105.0, 77.0, 43.0	0.07	62821	000483-77-2	91
20	21.865	Pentacosane	211.1, 169.1, 127.1, 99.0, 85.0, 71.0, 57.0, 43.0	0.13	185533	000629-99-2	86
21	22.082	3-Ethyl-5-(2-ethylbutyl)-octadecane	280.9, 127.0, 99.0, 71.0, 43.0	0.04	194509	055282-12-7	78
22	22.442	Dodecanoic acid	200.1, 157.1, 129.0, 101.0, 85.0, 73.0, 43.0	0.72	61120	000143-07-7	99
23	23.164	Diethyl phthalate	222.0, 177.0, 149.0, 121.0, 105.0, 75.9, 49.9	0.14	78782	000084-66-2	98
24	25.040	Heptacosane	197.0, 141.0, 113.0, 99.0, 85.0, 71.0, 57.0, 43.0	0.75	202662	000593-49-7	90
25	25.689	Tetradecanoic acid	228.2, 185.1, 128.9, 97.0, 73.0, 43.0	1.32	84455	000544-63-8	96
26	26.627	Caffeine	194.0, 165.1, 137.0, 109.0, 82.0, 67.0, 55.0, 42.0	0.42	56735	000058-08-2	95
27	27.277	Methyl hexadecanoate	270.1, 227.1, 185.1, 163.0, 143.0, 115.0, 97.0, 74.0, 57.0, 43.0	3.66	119400	000112-39-0	70
28	27.638	Dibutylphthalate	278.1, 223.0, 149.0, 104.0, 41.0	8.10	125786	000084-74-2	91
29	28.936	Linolenic acid	278.0, 222.1, 191.1, 163.1, 135.1, 108.1, 79.0, 55.0	30.57	30.57	000463-40-1	99
30	29.875	Tetratetracontane	127.0, 97.0, 57.0	9.36	241528	007098-22-8	91
31	30.668	2,2'-Methylenebis(6-tert-butyl-4-methylphenol)	340.2, 284.1, 228.0, 202.0, 177.1, 149.1, 121.0, 91.0, 57.0	13.37	177202	000119-47-1	99
32	31.029	Pentacosane	352.0, 169.0, 151.1, 113.0, 85.0, 57.0	1.93	185533	000629-99-2	91
Ethyl acetate extract							
1	3.754	Acetic acid butyl ester	100.9, 73.0, 43.0	6.55	8113	000123-86-4	72
2	4.548	Maleic anhydride	97.9, 54.0, 44.0	1.23	3034	000108-31-6	83
3	7.578	2H-Pyran-2,6(3 H)-dione	111.9, 83.9, 55.0, 39.0	0.51	6245	005926-95-4	58
4	8.155	Succinic anhydride	99.9, 56.0, 41.0	0.13	3595	000108-30-5	86
5	8.733	1-Methyl-2-pyrrolidinone	99.0, 84.9, 71.0, 44.0	5.05	3502	000872-50-4	91
6	12.413	Octanoic acid	144.0, 129.0, 115.0, 101.0, 85.0, 73.0, 59.9, 43.0	0.16	20837	000124-07-2	63
7	13.784	2,3-dihydrobenzofuran	120.0, 104.9, 91.0, 65.0, 39.0	0.26	9390	000496-16-2	86
8	15.299	3-Methyl-5-propylnonane	155.1, 141.1, 127.0, 112.0, 99.0, 85.0, 71.0, 57.0, 43.0	0.05	48914	031081-18-2	72
9	15.515	Eicosane	280.9, 155.1, 127.0, 112.9, 99.0, 85.0, 71.0, 57.0, 43.0	0.03	129492	000112-95-8	72
10	16.237	2-Methoxy-4-vinylphenol	150.0, 134.9, 121.0, 107.0, 90.9, 77.0, 63.9, 51.0, 37.9	0.11	24419	007786-61-0	59
11	17.247	2-tert-Butyl-4-methylphenol	164.1, 148.9, 134.9, 121.0, 105.0, 71.0, 77.0, 53.0	0.09	33649	002409-55-4	91
12	17.608	D-Allose	163.9, 128.9, 114.9, 98.0, 73.0, 60.0,43.0	0.07	45881	002595-97-3	64
13	18.618	Longifolene	205.1, 189.0, 160.9, 133.0, 107.0, 90.9, 67.0, 41.0	0.11	64270	000475-20-7	99
14	20.566	D-Allose	143.9, 98.0, 81.0, 60.0, 43.0	1.89	45881	002595-97-3	86
15	21.216	Butylated hydroxytoluene	220.1, 205.1, 191.1, 177.1, 145.0, 91.0, 57.0, 41.0	0.64	77555	000128-37-0	74
16	21.865	Tetratetracontane	169.0, 127.0, 97.0, 71.0, 41.0	0.07	241528	007098-22-8	63
17	22.659	Methyl-β-D-galactopyranoside	145.0, 127.0, 87.0, 59.9	0.83	56676	1000126-04-6	62
18	23.381	3-Undecen-5-yne	150.0, 134.9, 121.0, 107.1, 93.0, 79.0, 55.0, 41.0	0.15	23929	074744-27-7	70
19	24.102	2-Ethyl-4-methyl-1,3-dioxolane	86.9, 58.9, 31.0	0.37	8189	004359-46-0	62
20	25.112	2-Methyl-4-tert-butylphenol	166.0, 149.0, 121.0, 91.0,65.0, 41.0	0.36	33644	000098-27-1	60
21	25.690	Tetradecanoic acid	228.1, 211.1, 185.1, 157.0, 129.0, 97.0, 73.0, 43.0	1.32	84455	000544-63-8	99
22	26.700	Caffeine	194.0, 165.1, 136.0, 109.0, 67.0, 42.0	0.39	56740	000058-08-2	91
23	27.638	Tetradecanoic acid	185.1, 129.0, 97.0, 73.0, 43.0	8.47	84453	000544-63-8	59
24	28.143	3β-Octyloxirane-2β-dodecanoic acid	241.1, 211.1, 55.0	1.57	186877	003420-36-8	66
25	29.009	Octadecanoic acid	284.1, 241.1, 185.1, 97.0, 73.0, 55.0, 43.0	9.52	131262	000057-11-4	93
26	29.514	Hexadecanoic acid 2-hydroxyethyl ester	300.2, 270.0, 239.1, 209.1, 182.1, 154.1, 129.0, 104.0, 69.0, 43.0	3.56	144862	004219-49-2	58
27	30.668	2,2'-Methylenebis(6-tert-butyl-4-methylphenol)	340.2, 284.1, 228.1, 177.1, 149.1, 121.0, 91.0, 41.0	33.43	177202	000119-47-1	99

[a] Qual > 55 as potential existent compounds in the essential oils, and the detail information of each compound obtained was from the database of NIST11.L.

(6-tert-butyl-4-methylphenol) accounted for the highest percentage (33.43%), followed by octadecanoic acid (9.52%). Therefore, Cili fruit could potentially serve as a good dietary source of linoleic acid, octadecanoic acid, and 2, 2′-methylenebis (6-tert-butyl-4-methylphenol) for the prevention of certain chronic diseases, such as cancer and obesity.

3.5. Antibacterial activity of Cili fruit

Cili fruit extract exhibits a variety of biological properties (van der Westhuizen et al., 2008). However, there is no detailed study concerning its antibacterial effect. DIZ and MIC of Cili fruit aqueous-ethanolic extract were investigated using five *S. aureus* strains,

Table 5
Antibacterial effect of Cili fruit extract against *Staphylococcus aureus* strains.

Bacterial strains	DIZ (mm)		MIC	
	Cili extract (25 mg/mL)	Gentamicin (30 μg/mL)	Cili extract (mg/mL)	Gentamicin (μg/mL)
S. aureus SJTUF20857	17.6 ± 0.57^{b}	17.3 ± 0.57^{b}	6.25	6.25
S. aureus SJTUF20745	15.6 ± 1.15^{c}	15.7 ± 1.15^{b}	6.25	12.5
S. aureus SJTUF20758	22.3 ± 0.57^{a}	23.0 ± 0.00^{a}	6.25	0.78
S. aureus SJTUF20978	18.3 ± 0.57^{b}	17.3 ± 0.58^{b}	6.25	6.25
S. aureus ATCCF 25923	17.0 ± 0.05^{bc}	24.7 ± 0.58^{a}	6.25	0.78

Each measurement was determined in triplicate, and the results were expressed as mean ± SD. One-way analysis of variance (ANOVA) plus *post hoc* Tukey test was performed to compare means. Different superscript lowercase number indicated statistical significance ($p < 0.05$).

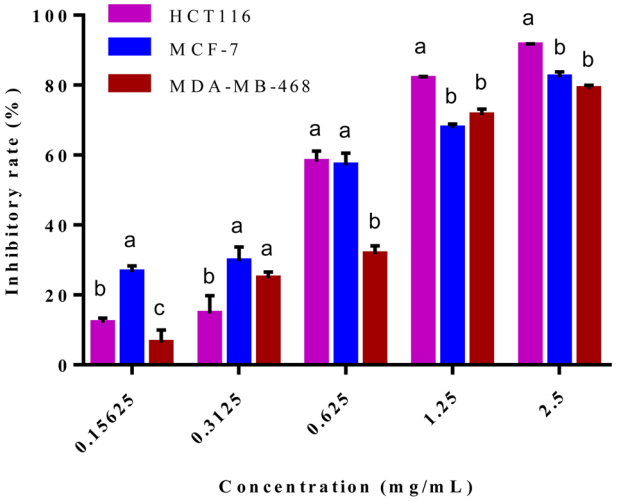

Fig. 1. The anti-proliferative effect of Cili fruit extract against cancer cells *in vitro*.

including four multidrug-resistant and one reference strains. Cili fruit extract overall exerted good antibacterial activities against both multidrug-resistant and reference strains of *S. aureus*, with DIZ values ranging from 15.6 to 22.3 mm, and MIC of 6.25 mg/mL (Table 5), indicating that Cili fruit was a good natural source of antibacterial agent, especially against drug-resistant bacteria. The data in this study suggested that Cili fruit might be a potential source as food preservatives and natural alternatives to antibiotics in animal feeding.

3.6. Anti-proliferative activity of Cili fruit

The anti-proliferative effect of Cili fruit aqueous-ethanolic extract was evaluated on three cancer cell lines, including MDA-MB-468 and MCF-7 human breast cancer cells, and HCT116 human colon cancer cells. As depicted in Fig. 1, Cili fruit aqueous-ethanolic extract displayed a good anti-proliferative potency with IC_{50} values ranging from 0.569 to 0.803 mg/mL towards three cancer cell lines, which might be due to its main anti-proliferative components, such as ascorbic acid, flavonoids, and proanthocyanidins. Recent high-profile preclinical studies have revealed that high-dose vitamin C can target many cancer cells by different mechanisms, including redox imbalance, epigenetic reprogramming, and oxygen-sensing regulation (Ngo et al., 2019). In addition, many flavonoids have been reported to inhibit the grow of cancer cells, such as catechins (Gan et al., 2018). Furthermore, proanthocyanidins have been reported to inhibit the proliferation of colorectal cancer cells by inhibiting the activity of adenosine triphosine triphosphate-binding cassette transporter proteins (Ravindranathan et al., 2018). Therefore, consumption of Cili fruit has the potential to prevent or inhibit the proliferation of cancer cells.

4. Conclusion

In summary, this study found that the wild fruit Cili (*Rosa roxburghii*) had high levels of ascorbic acid, total phenolics, and flavonoids, and exhibited a strong antioxidant activity, which was much superior to the selected common fruits and vegetables. Notably, we highlighted that ascorbic acid could significantly interfere the Folin-Ciocalteu method, leading to an overestimation of TPC in Cili fruit. This phenomenon could also exist while analyzing the TPC in some other plant extracts, particularly in those ascorbic acid-rich fruits and vegetables. Cili fruit had a large amount of linolenic acid and diverse phytochemicals, such as 2, 2′-methylenebis (6-tert-butyl-4-methylphenol), flavonoids, proanthocyanidins, and triterpenoids, and exhibited antibacterial and anti-proliferative effects. Therefore, Cili fruit is a good natural source of antioxidant phytochemicals and essential oils, and it should have wide applications in the modern food, nutraceutical, and cosmetic industries to develop functional products with potential health benefits.

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Author contributions

R.Y.G. conceived the idea of this study, Q.Q.Y., D.Z., A.K.F., and R.Y.G. performed the experiments, Q.Q.Y. and R.Y.G. wrote the draft, X.Y., J.R.Z., H.B.L., K.W.K., C.L.C., W.Y.L., H.C., and R.Y.G. edited and revised the manuscript. The final version was approved by all authors.

Declaration of Competing Interest

All authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2019.111928.

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