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Metabolite Profile, Antioxidant Activity and Anti-Candida Activity of Fermented Star Fruit Bioextract (*Averrhoa carambola* L.)

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Abstract

Nowadays bioextracts are widely used in various applications, however there is limited information on chemical constituents and biological activities of the bioextract produced for consumption. Therefore, the purpose of this study was to investigate the antioxidant activity, anti-candida activity and metabolic profile of fermented star fruit (*Averrhoa carambola* L.) bioextract. The fermented star fruit bioextract was produced through fermentation lasting 180 days, during which the activity and the metabolite profile were determined at 30-day intervals. The antioxidant activity was tested using the DPPH radical scavenging assay and the anti-candida activity was determined by the broth micro-dilution method. The metabolic profile was analyzed using LC-ESI-QTOF-MS/MS. The results showed that as fermentation proceeded, the potency of antioxidant and anti-candida activity observed from the bioextract increased. The lowest IC_{50} value for DPPH inhibition (25 %v/v) and the lowest minimum inhibitory concentration (6.25 %v/v) against *Candida albicans* were both observed after fermentation for 150 days. The metabolite analysis identified 4 main chemical classes: Sugars, organic acids, flavonoids and nitrogen compounds. The chemical diversity was highest in the Day 120 through Day 150 samples. This study shows that fermented bioextract from star fruit at incubation time 150 - 180 days carries potential health benefits for application in anti-candida and antioxidant products. Possible toxicity, skin and oral mucosal irritation should be considered for further study.

Keywords: Metabolite profile, Antioxidant activity, Anti-candida activity, Fermented bioextract, *Averrhoa carambola* L.

Introduction

Averrhoa carambola is a species of tree in the family Oxalidaceae that is native to Southeast Asia. This tree is commonly cultivated in tropical and semitropical regions for its edible fruits and various medicinal uses. The common name "star fruit" refers to the star shape of the fruit's cross-sectional slices. This tree's fruits and leaves have been widely used in Ayurvedic medicine to treat conditions such as cough, fever, mouth ulcer, sore throat, toothache, asthma, loss of appetite and constipation [1]. Previous phytochemical analysis of star fruits has indicated the presence of various saponins, alkaloids, flavonoids and tannins. Specifically, star fruit are known to contain proanthocyanidins and L-ascorbic acid, which are effective natural antioxidants [1,2]. Pharmacological studies on aqueous extracts of the fruit have demonstrated analgesic, hypoglycemic, hypocholesterolemia and hypolipidemic effects [3]. Antimicrobial activity against various pathogens has been reported from extracts of the stem, bark, fruit and leaf [1,4,5]. The fermented star fruit juice has previously demonstrated antimicrobial activity against various food borne pathogens [6]. Two concerns regarding a consumption of star fruit are that it is high in oxalic acid, which can potentially increase the risk of kidney stones, as well as caramboxin, which is a neurotoxin. Healthy people are therefore commonly advised against eating large quantities of star fruit, and people who are prone to kidney stones or have compromised renal function should avoid consumption of star fruit altogether [7].

The term "bioextract" or "fermented bioextract", previously introduced by Tancho [8], will refer here to any extract obtained from using a microorganism to aerobically or anaerobically ferment either plant or animal materials to enhance release of the chemical constituents of the fermented materials. Unlike alcohol drinks and vinegar, the fermented bioextract uses a variety of microorganisms as a starter and therefore it is similar to fermented plant extract, which is a plant functional food such as bean paste and natto [9]. As the microorganisms ferment the substrate, they also transform the chemicals that are present [8-10].

In Thailand, bioextracts are produced from plant waste for farming (particularly for organic farming) as a supplement to, or replacement for chemical fertilizers and insecticides [9]. There have been several reports, both in vitro and in the field, confirming the benefits of using such bioextracts for agriculture [11-13]. Various metabolites have previously been found in bioextracts, including saccharides, organic acids, amino acids, peptides, aroma compounds and antioxidant substances which makes bioextracts have its potential uses in agricultural aspects and also in health promotion aspects [9]. In addition, bioextracts can be used for the treatment of domestic wastewater [14]. Some other bioextracts in Thailand also been produced from fresh plants to be consumed for health benefits [15,16].

Bioextracts produced for consumption differ from those produced for agriculture or wastewater treatment in terms of the materials and production processes used. Consumable bioextracts are often based on fresh fruits that are selected for their particular nutritional values or medicinal properties. It is widely used as a drink supplement for promote health as well as for cosmetic purpose such as a facial toner and mouth rinses for oral thrush. Therefore, the production process needs to be well controlled to ensure safety [16]. Bioextracts for consumption, for example fermented noni juice, are widely used as nutritional supplements for purported health benefits [17,18]. The phytochemical profiling study on fermented fruit juice named Maha Bambad using DPPH antioxidant assay on-line couple with liquid chromatography found that hydrolysable tannins are responsible for the antioxidant activity of this fermented fruit juice [19].

However, research on the chemical constituents and biological activities of various consumable bioextracts has been very limited compared to the research on bioextracts for agriculture and waste water treatment purposes. The question of how long consumable bioextracts need to be fermented to maximize their benefits is also important to determine. Therefore, this study was aimed to investigate on metabolite profiles, antioxidant activity and in vitro antimicrobial activity against *Candida albicans* of the star fruit bioextract at different fermentation period. The overall aim of this research is to investigate the bioextract's potential as consumable health supplements or cosmetics.

Materials and methods

Preparation of the bioextract

The ripe fruits of star fruits (*Averrhoa carambola* L.) purchased from a fresh market in Phitsanulok, Thailand, were cut into small pieces approximately 1 cm^2. A total of 40 kg of star fruit pieces were transferred to a 100 L plastic barrel. A 25 g sachet of Por Dor 2 from Thailand's Department of Land Development was mixed into 10 L of tap water, which was then mixed into 10 kg of molasses. The combination of Por Dor 2, water and molasses was then added to the fruit pieces. The mixture was then left in a laboratory at a controlled temperature of 25 ℃. The mixture was allowed to ferment for 180 days, disturbed only twice a day for brief stirring. Every 30 days during the 180-day fermentation period, a 100-mL sample of the bioextract's liquid was removed via a faucet along with a strainer in order to measure the sample's physical and chemical properties, metabolic profiles as well as its levels of antioxidant and anti-candida activity.

Analysis of physical and chemical properties of the bioextract samples

The color and smell of each 30-day sample were recorded, along with measuring total alcohol content using an Ebulliometer (AllaFrance, France), total sugar content using a refractometer (Sato Keiryoki MFG, Japan) and pH using a pH-meter (Mettler Toledo, Switzerland). The total phenolic content was also measured using the Folin-Ciocalteu method [20]. Total phenolic content was expressed as μg of gallic acid equivalence (GAE)/mL of bioextract obtained from a calibration curve of gallic acid standard solution. All measurements were done in triplicate.

Analysis of metabolite profiles

The metabolite profiles of the star fruit bioextract samples were diluted 10 time and analyzed using Liquid Chromatography - Mass Spectroscopy coupled with electrospray ionization-quadrupole-time of

flight-mass spectrometry (LC-ESI-QTOF-MS/MS). The analysis was performed on a 6540 UHD Accurate-Mass-QTOF-LC/MS (Agilent Technologies, Palo Alto, CA, USA). The HPLC column was reversed phase Luna C-18(2), 4.6×150 mm^2, 5 µm (Phenomenex, USA). The mobile phases were water (A) and acetonitrile (B) both add 0.1 % formic acid. The elution steps were as follows: At 0 min 95:5 to 5:95 (A:B v/v) linear gradient in 30 min and hold on 10 min. Post time 5 min for column equilibrium before starting a new injection was employed. Column temperature 35 ℃, flow rate 0.5 mL/min, and injection volume 20 µL were used for all analyses. Mass spectra in the m/z range 100 - 1500 were obtained by negative ESI modes with a 250 ms/spectrum. The mass spectrometric conditions were as follows: Gas temperature 350 ℃, gas (N_2) flow rate 10 L/min, nebulizer gas pressure 30 psig, capillary voltage 3500 V, fragmentor potentials 100 V, Vcap 3500 V, Skimmer 65 V and Octopole RFP 750 V. The collision energy was used at 10, 20 and 40 V in the process of fragmentation. All the acquisition and analysis of data were performed using Agilent LC-MS-QTOF MassHunter Data Acquisition Software B.05.01 and Agilent MassHunter Qualitative Analysis Software B 06.0, respectively (Agilent Technologies, USA). The identification was proposed from the unique fragmentation pattern and public database comparison.

Antioxidant activity testing

Antioxidant activity of the bioextract samples was tested using the DPPH radical scavenging assay. The assay was performed according to methodology described by Brand-Williams *et al.* [21]. Bioextract sample was prepared in ethanol at different concentrations of 3.12, 6.25, 12.50, 25.00 and 50.00 %v/v. A 0.5 mL of diluted bioextract sample, 3.0 mL of absolute ethanol and 0.3 mL of 0.5 mM DPPH radical solution in ethanol were combined. After allowing a reaction time of 30 min at room temperature in dark place, unconverted DPPH is detected by a UV-VIS spectrophotometer (Beckman, CA, USA) at 517 nm against blank and percent inhibition was calculated. The scavenging activity percentage (AA %) was determined according to the method described by Mensor *et al.* [22]. Activity of the sample was calculated from [(A1 − A2)/A1]×100, where A1 is the absorbance of the control, and A2 is the absorbance of the standard or the sample. Gallic acid was used as standard free radical scavenger and activity of the bioextract was compared with it. The 50 % Inhibitory Concentration (IC_{50}) values were obtained from prepared inhibition curves. All determination was carried out in triplicate.

Anti-candida activity testing

Anti-candida activity of the bioextract samples against *Candida albicans* ATCC10231 was determined using the broth micro-dilution method [23]. The bioextract samples (100 µL) were 2-fold serially diluted with RPMI 1640 medium in 96-well microtiter plates to obtain respective concentration ranges of 3.13 - 50.00 %v/v. The *Candida* suspension was added to each well. A growth control was created by combining RPMI 1640 medium and the *Candida* suspension. A no growth control was created by serially diluting bioextract with media as described above, but without adding any *Candida* suspension. The plates were incubated at 37 ℃ for 48 h. The growth in each well was estimated by measuring well turbidity with the spectrophotometer set at 600 nm absorbance. The minimum inhibitory concentrations (MICs) were defined as the lowest concentration that exerted 90 % growth inhibition compared to the growth control. The minimum fungicidal concentration (MFCs) was defined as the lowest concentration of wells that had no microbial growth when 10 µL of the well contents was plated on potato dextrose agar and then incubated at 37 ℃ for 48 h. All experiments were repeated in triplicate. The MICs and MFCs are shown as representatives from a single experiment.

Data analysis and statistics

All experiments were carried out in triplicate except the LC-ESI-QTOF-MS/MS analysis. The data were presented as mean ± standard deviation except for MICs and MFCs. The MICs and MFCs were shown as representatives from a single experiment. One-way analysis of variance (ANOVA) and Duncan multiple comparison tests at the significance level of $p < 0.05$ were applied using the SPSS 17.0 for Windows Software Package.

Results and discussion

Physical and chemical characteristics

The fruits of *Averrhoa carambola* L., which are relatively abundant and inexpensive in the tropics, was used to prepare bioextracts with 180-day fermentation period. During the 180 days of fermentation, the bioextract samples were clear brownish solutions with a pleasant fruity smell. The changing

characteristics of the star fruit bioextract over the course of the 180-day fermentation period are shown in **Table 1**. The alcohol contents initially rose quickly to 8.00 %v/v on Day 30, and then significantly decreased until leveling out at 3.00 %v/v from Day 120 onward. The sugar contents were ranged from 20.00 °Brix on Day 0 to 6.09 °Brix on Day 180. The sugar contents were significantly decreased from 20.00 °Brix on Day 0 to 7.00 °Brix on Day 90 and subsequently the contents were equalized till Day 150 before leveling down significantly to 6.09 °Brix on Day 180. The initial pH of the star fruit bioextract was 5.67, which then fell fairly steadily, ending at 3.87 on Day 90. After that, the pH was ranged from 4.17 - 4.10 from Day 120 to Day 180.

°Brix and pH of the star fruit bioextract were both followed to monitor the fermentation process. The initial %Brix of 20.00 and pH of 5.67 are consistent with similar values reported in previous studies on the production of fermented star fruit beverages [24,25]. During fermentation of the star fruit alcoholic fermented beverage, which utilized *Saccharomyces cerevisiae* as a starter culture, °Brix fell rapidly from approximately 20.00 to 6.00 during the first 10 days, and pH went from 4.00 - 5.00 down to 3.74 - 4.35 during the same period [24]. However, the °Brix in the current study fell less quickly, although the pH reached a similar 4.50 after 30 days of fermentation. The current study's bioextract alcohol content increased to 8.00 % within 30 days of fermentation, while the beverage fermentation studies' alcohol content rose to around 12 % within 4 days of fermentation and then remained at that same level [24]. In another study, the *Lactobacillus* spp. were used and the results showed that the °Brix and pH were decreased slightly on Day 8 of fermentation [24]. These differences could be resulted from several possible factors, for example the respective varieties of metabolites present in the different fermentations, the different raw materials used initially, different starter microorganisms, and different fermentation conditions [24-26]. For example, the beverage star fruit study utilized *S. cerevisiae*, a species of yeast commonly used to ferment alcoholic beverages [24], while the current study utilized Por Dor 2, which contains a variety of microorganisms and is intended specifically for producing bio-fertilizer.

Total phenolic content began at 216.67 μg GAE/mL on Day 0 and rose significantly during the first 30 days to 908.33 μg GAE/mL, after which it continued to rise at a slower rate until reaching 1164.24 μg GAE/mL on Day 180, as shown in **Table 1**. Several previous studies have indicated that various conditions during the fermentation process, such as temperature and amount of raw materials, can significantly affect total phenolic content as well as antioxidant activity [26,27]. It is likely that most of these effects can be explained by how these conditions influence the metabolic activity of microorganisms are used as the starter culture.

Table 1 Chemical characteristics of the star fruit bioextract at different time points during fermentation.

Fermentation Days	Alcohol (%v/v)	°Brix (%w/w)	pH	Total phenolic contents (μg GAE/mL)*
0	0.00 ± 0.00^{a}	20.00 ± 1.09^{e}	5.67 ± 0.02^{d}	216.67 ± 3.37^{a}
30	8.00 ± 0.09^{e}	16.00 ± 0.25^{d}	4.50 ± 0.11^{c}	908.33 ± 10.75^{b}
60	4.50 ± 0.05^{d}	8.05 ± 0.09^{c}	3.87 ± 0.03^{a}	977.22 ± 4.83^{c}
90	3.50 ± 0.05^{c}	7.00 ± 0.24^{b}	4.17 ± 0.03^{b}	985.28 ± 24.66^{c}
120	3.00 ± 0.10^{b}	6.98 ± 0.15^{b}	4.16 ± 0.07^{b}	1074.85 ± 3.94^{d}
150	3.00 ± 0.17^{b}	6.93 ± 0.13^{b}	4.10 ± 0.03^{b}	1103.03 ± 20.43^{e}
180	3.00 ± 0.09^{b}	6.09 ± 0.27^{a}	4.10 ± 0.07^{b}	1164.24 ± 23.93^{e}

Different lowercase letters in the same column indicate the statistical difference between the mean values ($p < 0.05$). *μg of gallic acid equivalence/mL of bioextract

Antioxidant and anti-candida activities

In this current study, the antioxidant activity was investigated for a potential of application in cosmetic aspects and the antimicrobial activity against *Candida albicans,* a common pathogen caused oral thrush, was explored for a possible application in oral hygiene products. The antioxidant and anti-candida activities of the star fruit bioextract at different days during fermentation are shown in **Table 2**. The

bioextract samples scavenged DPPH radicals strongly, with IC_{50} values ranging from 45.10 to 49.90 %v/v or 104.71 to 124.27 μg GAE/mL. The 150-day bioextract sample demonstrated the highest scavenging capacity, with an IC_{50} of 45.10 %v/v and 124.27 μg GAE/mL. The anti-candida activity increased during the fermentation period. The minimum inhibitory concentration (MIC) values for *C. albicans* ranged from 6.25 to 50 %v/v, and the minimum fungicidal concentration (MFC) values for *C. albicans* ranged from > 50 to 25 %v/v. The highest anti-candida activity was observed during 150-day and 180-day bioextract fermentation, which had the MIC of 6.25 %v/v and the MFC of 25 %v/v. The highest activities during 150-day and 180-day bioextract fermentation related with the highest total phenolic contents at the same period of fermentation.

Most of previous studies investigating antimicrobial activities of star fruit (*Averrhoa carambola* L.) focused on their stem, bark and leaf extracts and few studies were explored on its fruits and juices. The study of dried fruit methanol extracts revealed that the extracts had no activity against *Candida lipolytica*, but it had potent antioxidant activity [28]. Preliminary screening for antimicrobial activity of the fermented star fruit juice has previously demonstrated the activity against various food borne pathogens, including *Campylobacter jejuni*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Escherichia coli* [6].

Several studies have shown that the star fruit is a good antioxidant source [29]. Another previous study showed that the fruit residues had much higher total phenolic content and DPPH radical scavenging activity than the star fruit's juice [30]. These previous data suggest that fermenting small pieces of whole star fruit should probably yield higher antioxidant activity than fermenting just star fruit juice. Nevertheless, the current study showed that the 150-day and 180-day star fruit bioextract exhibited strong anti-candida and antioxidant activities with the MIC of 6.25 %v/v, the MFC of 25 %v/v and IC_{50} of 124.27 μg GAE /mL. The process of fermentation is already known to transform organic material into useful products, including bioactive compound [31,32], and this could explain the antioxidant and anti-candida activities found in this star fruit bioextract. Therefore, the star fruit bioextract is a potential resource for both antioxidant and anti-candida applications. More studies on the anti-candida activity against various species and strains of *Candida* spp. as well as antibiotic resistant strains are needed in order to further develop effective health products from the bioextracts.

Table 2 Antioxidant activity (50 % Inhibitory Concentration, IC_{50}) and anti-candida activity (MIC and MFC) of the star fruit bioextract sampled at 30-day intervals during the fermentation process.

Days past during fermentation process	50 % Inhibitory Concentration (IC_{50})		MIC	MFC
	%v/v bioextracts	μg GAE/mL*	%v/v	%v/v
30	49.90 ± 0.38^{d}	104.71 ± 0.80^{a}	50.00	> 50.00
60	48.67 ± 0.45^{c}	107.36 ± 1.00^{b}	50.00	> 50.00
90	49.13 ± 0.11^{d}	110.96 ± 0.25^{c}	12.50	> 50.00
120	47.66 ± 0.39^{b}	116.70 ± 0.95^{d}	12.50	> 50.00
150	45.10 ± 0.25^{a}	124.27 ± 0.65^{e}	6.25	25.00
180	47.68 ± 0.18^{b}	116.87 ± 0.43^{d}	6.25	25.00

Different lowercase letters in the same column indicate the statistical difference between the mean values ($p < 0.05$). *μg of gallic acid equivalence/mL of bioextract

Metabolite profiles

Metabolite diversity profiles of the star fruit bioextract sampled at 30-day intervals during 180-day fermentation are shown in **Figure 1** as total ion chromatograms of LC-ESI-QTOF-MS/MS. The diversity of metabolites increased from Day 60 to Day 150 and then by Day 180 it started decreasing.

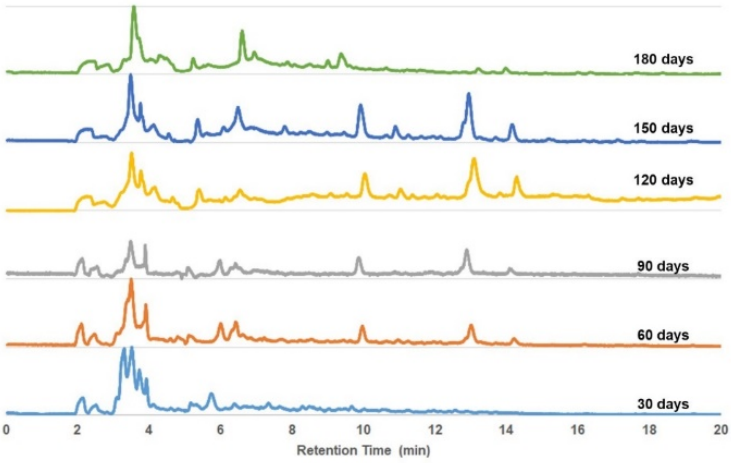

Figure 1 Total ion chromatogram of LC-ESI-QTOF-MS/MS show metabolite diversity levels of the star fruit bioextract sampled at 30-day intervals during fermentation. The chromatogram displayed in 20 min of 40min. (Y axis is electronic count and X axis is retention time).

Numerous metabolites were found in the star fruit bioextract, as identified from the LC-ESI-QTOF MS/MS data, and these metabolites are shown in **Table 3**. Four main chemical classes were found in the bioextract: Sugars, organic acids, flavonoids and nitrogen compounds. Chemical diversity was highest in the Day 120 through Day 150 samples. By Day 180, comparatively few chemicals remained. More specifically, sugars contained in the Day 30 sample were glucose, sucrose, fructose and acetyl-maltose. By Day 150, all of these had been consumed by the microbes except for glucose, which then remained alone. This result was agreed with the previous study on fermented star fruit juce by *Lactobacillus* strains, that sucrose and frutose were depleted while glucose remained at the end of fermentation [25]. Nevertheless, a recent study shows that genotype, ripening stages, growth conditions and agricultural practice influence on metabolic diversity profile of star fruit fruits [33].

Regarding the organic acids, 7 organic acids were found in the bioextract at Day 30: Quinic acid, citric acid, succinic acid, citraconic acid, dehydroascorbic acid, valeric acid and malic acid. Of those, only valeric acid remained by Day 60, at which point a new variety of organic acids was detected, namely: Ascorbic acid, lipoic acid and pisidic acid. All of this second group remained through the Day 150 sample. At Day 180, there were 3 kinds of organic acid left: Ascorbic acid, shikimic acid and propanoic acid. These changes in organic acids could be the result of several possible pathways. For example, citrate fermentation via citrate lyase can transform citric acid into other organic acids or flavor compounds. Also, malic acid which is the most abundance organic acid in fresh star fruit fruits [25] can be removed, for instance, via the malolactic reaction [34].

Four flavonoids were present in the extract sample at Day 30, and all 4 of these subsequently disappeared. Then 5 other flavonoids were found at Day 60. By Day 150, all of these were also gone except for apigenin, which is a versatile naturally bioactive compound, with antimicrobial and antioxidant properties, among others. At Day 180, a single flavonoid, trimethoxyflavone, was present.

Concerning nitrogen compounds, derivatives of 4 amino acids were found at Day 30, all of which, except for the derivative of serine, were gone by Day 120. From then, derivatives of phenazine, benzamide and other compounds appeared and remained through Day 150. Additionally, at Day 60, 4 fragrant compounds were found: Methyl (r)-3-methyl-2-oxopentanoate, tetrahydroxybenzene, pyrocatechol and mesifurane. These fragrant compounds have never been identified in fresh star fruit. The fermentation process transformed the fresh star fruit into a unique pleasant smelling bioextract with a scent that would be agreeable for use in health care products.

There is a limitation in this current study that could be addressed in future research. Previous studies have shown that methanol, a toxic alcohol, was produced during bioextract fermentation processes [35]. In this study, the total alcohol content was measured by Ebulliometer. The method is accurate for simple mixtures of alcohol and water however it cannot identify types of alcohol in samples [36]. Even though, this current study measured metabolite profile by using the LC-ESI-QTOF MS/MS, it cannot measure volatile substances. Therefore, the headspace gas chromatography with flame-ionization detection should be employed to identify of alcohols and other volatile substances that may be produced during this fermentation process.

Table 3 Metabolite compounds found in star fruit bioextract sampled at 6 times during fermentation (+ = present and − = absent).

RT(min)	m/z[M-H]-	Metabolite compounds	Days of fermentation					
			30	60	90	120	150	180
Sugars								
3.27	179.0631	Glucose	+	+	+	+	+	+
3.37	683.2401*	Sucrose	+	−	−	−	−	−
3.54	179.0631	Fructose	+	+	+	+	+	−
3.73	383.1303	Acetyl-maltose	+	−	−	−	−	−
Organic acids								
3.58	359.1278	Deoxyloganic acid	−	−	−	−	−	+
3.74	191.0635	Quinic acid	+	−	−	−	−	−
3.85	237.0636	3-Deoxyoctulosonic acid	−	+	−	−	−	−
3.88	207.0528	Lipoic acid	−	+	+	−	−	−
3.89	119.036	2,4-dihydroxy-butanoic acid	−	+	−	−	−	−
4.25	191.0211	Isocitric acid	−	+	+	+	−	−
4.55	147.0307	Citramalic acid	−	−	−	−	+	−
5.20	147.0311	L-threo-3-Methylmalate	−	+	+	+	+	−
5.43	133.0506	2,3-dihydroxy-3-methylbutyric acid	−	−	+	+	−	−
5.63	235.0469	2-C-(2-Carboxyethyl)-3-deoxypentaric acid	−	−	−	−	+	−
5.74	191.0271	Citric acid	+	−	−	−	−	−
6.01	159.0675	2-Propylsuccinic acid	−	+	+	+	+	−
6.39	117.0255	Succinic acid	+		+	−	−	−
6.44	175.0262	L-Ascorbic acid	−	+	+	+	+	+
7.35	129.0256	Citraconic acid	+	+	+	−	−	−
7.35	173.0163	Dehydroascorbic acid	+	−	−	−	−	−
8.08	173.0474	Shikimic acid	−	−	−	−	−	+
8.55	141.02	Muconic acid	−	−	−	−	+	−
9.40	255.0515	(2R,3S)- Piscidic acid	−		+	+	+	−
10.02	117.0613	3-hydroxy valeric acid	+	+	+	+	+	−
9.99	181.0522	(R)-3-(4-Hydroxyphenyl)lactate	−	+	+	+	+	−
10.63	353.0886	Chlorogenic Acid	−	−	+	+		−
10.97	175.0626	3-propylmalic acid	+	+	+	+	+	−
11.62	137.0254	Salicylic acid	−	−	−	−	+	−
13.01	131.0722	5-hydroxy caproic acid	−	+	+	+	+	−
14.22	165.0569	3-(2-Hydroxyphenyl) propionic acid	−	+	+	+	+	+
Flavonoids								
4.81	333.062	6-Methoxytaxifolin	−	+	−	−	−	−
6.94	327.0905	7-hydroxy-3,4',8-trimethoxyflavone	−	−	−	−	−	+
8.29	329.0974	Eriodictyol 7,3',4'-trimethyl ether	+	−	−	−	−	−
9.02	315.0816	5,7-Dihydroxy-8,4'-dimethoxy Flavanone	+	−	−	−	−	−
9.31	315.0819	7,4'-Dihydroxy-5,2'-dimethoxy isoflavanone	+	−	−	−	−	−
9.68	361.1245**	7-Hydroxy-2',4',5'-trimethoxy isoflavanone	+	−	−	−	−	−
11.23	563.1455	Apigenin7-O-[β-D-apiosyl-(1->2)-β-D-glucoside]	−	+	+	+	+	−
11.84	461.1114	Quercetin 3-methyl ether 7-rhamnoside	−	−	−	+	+	−
13.83	373.1394	(2S)-5,6,7,3',4'-Pentamethoxy Flavanone	−	−	+	−	−	−
Nitrogen compounds								
3.81	353.0825	(S)-5-amino-2-(3-(2-amino-2-oxoethoxy)-4-nitrobenzamido)-5-oxopentanoic acid	−	−	−	+	−	−
3.54	359.1298	Dityrosine	+	−	−	−	−	−

RT(min)	m/z[M-H]-	Metabolite compounds	Days of fermentation					
			30	60	90	120	150	180
3.944	281.0967	2-Aminoadenosine	+	−	−	−	−	−
4.132	294.0918***	Glutaminyl hydroxyproline	−	−	−	+	−	−
5.256	290.0904	2-Deoxy-2,3-dehydro-N acetylneuraminic acid	−	+	−	−	−	−
5.336	290.0977**	Asparaginyl-Hydroxyproline	+	−	−	−	−	−
7.031	317.0583	2-[(8-Oxo-1,8-dihydroindeno[1,2-d]imidazol-2-yl)carbonyl] benzoic acid	−	−	−	−	−	+
8.49	296.0832	1-Phenylisoxazolo [4,3-a]phenazine	−	−	−	−	−	+
8.62	402.1148	2,4-Dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazin-3(4H)-one 2-glucoside	−	−	−	+	−	−
8.93	240.052	N-(2,3-Dihydroxybenzoyl)-L-serine	+	−	+	+	+	+
10.72	252.059	2-Acetamido-6H-dibenzo[b,d]pyran-6-one	−	−	+	−	−	−
Fragrant compounds								
7.88	143.072	Methyl (r)-3-methyl-2-oxopentanoate	−	−	−	−	−	+
8.60	141.0255	1,2,3,5-Tetrahydroxybenzene	+	−	−	−	−	−
9.37	141.0567	Mesifurane	−	−	−	−	−	+
11.93	109.0301	Pyrocatechol	−	−	+	+	−	−

*dimeric form [2M-H]- **formate adduct [M+HCOO]-*** chloride adduct[M+Cl]-

Conclusions

This study indicates that star fruit bioextract could be used as a source of anti-candida and antioxidant activity for development health promotion and cosmetic products. The 150-day and 180-day star fruit bioextract exhibited strong anti-candida and antioxidant activities. The concentration of 6.25 %v/v can inhibit the growth of *Candida albicans*. Based on DPPH radical scavenging, 1 mL of the star fruit bioextract has DPPH scavenging capacity equal to that of gallic acid at 124.27 μg. Therefore, the results also suggest that, in order to produce star fruit bioextract of greatest effectiveness, the mixture should be fermented for an optimal duration of approximately 5 months, at which time it has the highest diversity of metabolites, the highest concentration of total phenolics and the highest levels of anti-candida and antioxidant activities. LC-MS-based metabolite profiling proved to be applicable and useful for analyzing this bioextract. Moreover, at each stage of fermentation which phytochemical present can be used for other health benefits. Thus, the bioextract has a potential for development of consumable health supplements or cosmetics used in the oral cavity or on the skin. Any potential for skin and oral mucosa irritation or toxicity, as well as its general efficacy in vivo, should be further studied.

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