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In vitro evaluation of bioactive properties of banana sap

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Abstract

Banana sap is currently designated as a waste subsequent to utilization of pseudo stem in pulp and paper industry as well as other applications which is contributing to the environmental pollution. In the present study, banana sap and its crude extracts were evaluated for antimicrobial, antioxidant and anticancer properties. The role of oxidized and un-oxidized banana sap for its antimicrobial potential against a microbial test panel comprising gram positive as well as gram negative bacteria and *Candida albicans* using *in vitro* micro broth dilution assay. The un-oxidized banana sap exhibited a significantly higher antibacterial potential as evident by a lower minimal inhibitory concentration (MIC) ranging between 15.625 to 62.5 mg/mL. *In vitro* radical scavenging activity of dichloromethane (DCM) extract of banana sap by DPPH method exhibited 54.62 ± 1.09 (µg/mL) IC_{50} value at the concentration of 1 mg/mL. Dichloromethane extract of banana sap showed maximum cytotoxic effect with human breast cancer (MCF-7) cell proliferation at the concentration of 100 µg/mL which was $78.37 \pm 0.05\%$ and the cytotoxic effect significantly increased with increasing concentration of banana sap extract. Furthermore, LCMS studies revealed the presence of bioactive compounds in dichloromethane extract of banana sap, such as rescinnamine derivative, dihydrorescinnamine and epimedin A. The present study suggested that banana sap is a promising source of bioactive compounds with relevant antimicrobial, antioxidant and anticancer properties.

Keywords Banana sap · Scavenging ability · Antimicrobial activity · Cytotoxic · LCMS · Phytochemical constituents

Abbreviations

LCMS	Liquid chromatography mass spectrometry
DCM	Dichloromethane
MIC	Minimum Inhibitory Concentration
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
TAA	Total antioxidant activity
NaCl	Sodium chloride
$Na_2S_2O_5$	Sodium Pyrosulfite
EDTA	Ethylenediamine tetraacetic acid
DMSO	Dimethyl Sulfoxide
MH	Muller Hinton
SDA	Sabouraud Dextrose Agar
EUCAST	European Committee on Antimicrobial Susceptibility Testing
TTC	2, 3, 5-Triphenyl tetrazolium chloride
NCCS	National Centre for Cell Sciences
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal Bovine Serum
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
ELISA	Enzyme Linked Immuno Sorbent Assay
OHRLCMS	Orbitrap Liquid Chromatography Mass Spectroscopy
IC_{50}	Inhibitory Concentration $_{50}$
Minutes	Min
Hours	H

Introduction

Plant-derived natural products have played a key role in the process of drug discovery and development ever since the advent of modern medicine. The diverse chemical scaffolds present in plants exhibit a spectrum of biological activities and thus have been the mainstay for the development of drugs that have been classified as semi-synthetic or natural product derived. They have proven to be used for the development of effective therapeutic interventions in the treatment of cancer,

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diseases caused by multi-drug resistant bacteria and viruses apart from curing immunity-associated disorders (Majhi and Das 2021). Banana is a flowering plant that belongs to *Musa* spp. It is grown in the tropical regions of the world primarily for its fruits and contributes an important food source after rice, wheat, and maize. Traditionally and scientifically, banana has been found to contain medicinal properties (Nadumane and Timsina 2014). Different parts of the banana such as pseudo-stem, leaves, sap, and flowers have been documented to possess medicinal or curative properties such as anti-snake venom (Borges et al. 2005) anti-gastric ulcer (Khamboonruang et al. 2015), antimicrobial (Budi et al. 2020), antidepressant (Kar et al. 2019), antihypercholesterolemic (Dikshit et al. 2016), antioxidant effect (Dikshit et al. 2016), antidiarrheal (Yakubu et al. 2015) and antidiabetic activity (Sheng et al. 2017). After harvesting the bananas, the plant dies. The dead plants comprising of leaves, pseudo-stems contribute to huge agro-waste from the banana farms into the environment (Gupta et al. 2019). Moreover, the pseudo-stem of banana is finding application in the paper manufacturing industry along with a wide range of value-added products such as ropes, mats etc. However, the immediate concern is the huge amount of sap present in the pseudo-stem of the plant (Gupta et al. 2022). Previous studies on the phytochemical composition of banana sap have indicated the presence of compounds comprising of hydrocinnamic acids, flavones, and flavonoids (broadly phenolics) (Pothavorn et al. 2010). More specifically, stem juice of *Musa paradisiaca* reported the presence of antidiabetic compounds such as lupeol, ferulic acid, vanillic acid, trans-cinnamic acid, p-hydroxybenzoic acid, p-coumaric acid, rutin, catechin/ epicatechin, chlorogenic acid, gallic acid, caffeic acid, and nicotiflorin (Nguyen et al. 2017). The banana stem juice is contributing as liquid waste and is an environmental concern as it is being disposed of in the environment after the utilization of the banana stem for different applications (Gupta et al. 2022). Phytochemical studies on banana stem juice/ sap have been initially carried out by Pothavorn et al. (2010) and Nguyen et al. (2017) for possible exploitation of the banana stem juice. Based on this premise, the present investigation was undertaken to explore the bioactive potential of the banana stem sap by fractionating with solvents and analysing their antioxidant, anticancer and antimicrobial activities. Further, the composition of these solvent extracts were analysed by LC–MS to find out the phytochemicals which possibly would be providing the medicinal potential to the banana sap.

Materials and methods

Preparation of extracts

Fresh banana pseudo-stem of Grand naine cultivar was collected from Thapar Institute of Engineering and Technology Campus, Patiala, India. Banana stem juice was extracted from the stems with the help of local market. The sap from the banana pseudo-stem was extracted with the help of a commercial squeezer in the local market. Prior to extracting the sap, the squeezer was thoroughly washed and disinfected. Subsequent to the extraction of juice, banana sap was concentrated to ten times with the help of rotary evaporator. The concentrated sap was further extracted with dichloromethane (DCM). One hundred (100) mL of concentrated banana sap was mixed three times with 70 mL of DCM to obtain the DCM residue with the help of solvent extraction process at 35 ºC (Vasundhara et al. 2017). Further, the solvent was removed by rotary evaporator (IKA RV 10) and extract was dried by using lyophilizer. The obtained dried residue was dissolved in dimethyl sulfoxide (DMSO). DCM extract was used for the evaluation of anticancer, antioxidant activities and LCMS analysis.

Antioxidant assay

The antioxidant capacity of the dichloromethane extracts of banana sap was carried out via free radical scavenging effect (Sharma et al. 2017). In 96 well micro titer plate, 50 µL of banana sap extracts in increasing concentrations (100, 250, 500 and 1000 µg/mL) were mixed with 150 µL of DPPH (100 µM) in methanol. Ascorbic acid (Stock solution: 100 µg/mL; Working volume: 4µL) was used as the positive control. The microtitre plate was incubated in the dark for 45 min and subsequently absorbance was recorded at 517 nm using microplate reader (Tecan infinite, Austria).

The scavenging activity was expressed as:

$$\text{Scavenging activity } (\%) = \{(A_{control} - A_{sample})/A_{control}\} \times 100$$

Preparation of un-oxidized banana sap

80% ethanol containing 100 mM NaCl, 0.2 mM ascorbic acid, 40 mM citric acid, 0.1 mM $Na_2S_2O_5$, 0.25% Triton X-100 and 0.2 mM EDTA was prepared in amber coloured bottle (Pothavorn et al. 2010). Stems were processed to make sap. To prevent oxidation, freshly extracted sap was mixed with 80% ethanol solution in the ratio of 1:1. The sap was then heated at 80ºC for 45 min and subsequently centrifuged (Thermo Fischer Scientific, Sorvall Legend XFR Centrifuge, 75004538, Germany) at 11250 × g for 15 min at 25˚C. The supernatant was collected and concentrated in rota evaporator. Subsequently it was lyophilized and dissolved in 10% DMSO (dimethyl sulfoxide) and stored at 4 °C until further use.

Test microorganisms

The microbial test panel comprised of *Escherichia coli* ESS 2231, *Bacillus megaterium* FH 1127, *Pseudomonas aeruginosa* M35, *Staphylococcus aureus* ATCC 33591 and *Candida albicans* ATCC10231 used for the study of the anti-candidal activity. The bacterial cultures were individually streaked on Muller Hinton (MH) agar plate and incubated overnight and subsequently transferred a single colony aseptically to 100 mL of pre-sterilized MH broth and incubated at 37 °C overnight. In case of *Candida albicans*, it was streaked on Sabouraud Dextrose Agar (SDA) plate and incubated overnight. A single colony was picked from this plate and transferred to Sabouraud Dextrose broth and incubated at 37 °C prior to test.

In vitro antibacterial assay

Antibacterial activity of the oxidized and unoxidized extract of the banana sap was determined through *in vitro* microbroth dilution assay (Jorgensen et al. 1999; EUCAST 2018). Briefly, 96-well microtiter plate was used to evaluate the antibacterial activity and determine visual minimal inhibitory concentration (MIC) determination. The oxidized and unoxidized extract of the banana sap was dissolved in 10% dimethyl sulfoxide (DMSO) and tested in a concentration from 500 mg/mL to 1.95 mg/mL i.e., 2-fold serial dilutions. Fifty (50) µL of the 0.5 McFarland adjusted bacterial suspension in saline was added to 125 µl of MH broth to achieve a final bacterial cell concentration of 10^5 cells in the well. Amoxicillin (0.1 mg/mL) was used as a positive control. The titer plate was incubated at 37 °C for 24 h. After 24 h, 20 µL of 0.02% of TTC (2, 3, 5-triphenyl tetrazolium chloride) was used for the visualization of the MIC.

In vitro anti-candidal activity

The anti-candidal activity of the oxidized and unoxidized of the banana sap extract was evaluated by *in vitro* microbroth dilution assay (Jorgensen et al. 1999; EUCAST 2018). Briefly, 96-well microtiter plate was used to evaluate the anti-candidal activity as well as to visualize minimal inhibitory concentration (MIC). The banana sap extract was dissolved in 10% dimethyl sulfoxide (DMSO) and tested in a concentration from 500 mg/mL to 1.95 mg/mL i.e., 2-fold serial dilutions. Fifty (50) µl of the 0.5 McFarland adjusted suspension of *Candida albicans* in saline was added to 125 µl of MH broth to achieve a final concentration of 10^5 cells in the well. Fluconazole (0.1 mg/mL) was used as a positive control. The titer plate was incubated at 37 °C for 24 h. After 24 h, 0.01% of Resazurin was used for the visualization of the MIC.

Maintenance of cell lines

Human breast cancer (MCF-7) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. The cell line was maintained in complete Dulbecco's Modified Eagle Medium (DMEM) in the humidified incubator with 5% CO_2 in 37 °C in T25 flasks. Complete medium means DMEM supplemented with 10% (v/v) FBS, 100 IU/mL penicillin, 100 µg/mL streptomycin and 2.5 µg/mL amphotericin.

Cell growth inhibition assay

The growth effect of dichloromethane extract of banana sap on MCF-7 cells was measured by 3-(4, 5 dimethylthiazol-2-yl)-2–5 diphenyl tetrazolium bromide (MTT assay) (Lohia and Baranwal 2017). 2×10^4 cells MCF-7 cells were seeded in 96-well microtiter plates and kept overnight in the incubator. The plate was incubated at 37 °C in a humidified incubator maintained at 5% CO_2 (New Brunswick Galaxy; Eppendorf, Hauppauge, NY, USA). After incubation, the extract was added in increasing concentration (5, 10, 25, 50, 75 and 100 µg/mL) to the wells. After 72 h incubation of plates, 20 µL MTT (5 mg/mL) was added in each well and again incubated for 4 h. 100 µL DMSO was added to each well to dissolve the formazan crystals. The absorbance was recorded at 570 nm taking reference wavelength at 620 nm on ELISA plate reader (Tecan Infinite, Groedig, Austria, Pro ELISA reader). Paclitaxel was used as the positive control at a concentration of 20 µg/mL (Working volume: 4 µL). Cell growth inhibition was expressed as:

$$\text{Cell growth inhibition } (\%) = \{(A_{\text{untreated}}\text{cell} - A_{\text{treated}})/A_{\text{untreated}}\} \times 100$$

LC–MS analysis

The organic layer of DCM was further rota-evaporated and stored in vials at 4°C until further use and sent to SAIF, IIT Bombay, for Orbitrap LCMS (OHRLCMS) analysis. The column used for orbitrap LC–MS was Hypersil gold 3 micron 100 × 2.1 MM with run time 30 min on instrument (VANQUISH on IITB_QE-PC). 5 µl of DCM sample gradient was injected for the analysis.

Statistical analysis

All the experiments were performed in triplicate. The data were analyzed by one way ANOVA (analysis of variance) and

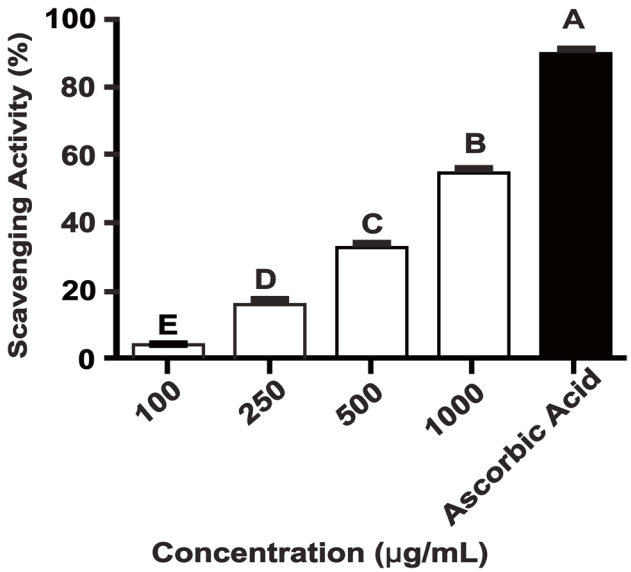


Fig. 1 Antioxidant potential of dichloromethane extract of banana sap. Mean followed by same letter are not significantly different

the means were compared by Tukey's test at $p < 0.05$ using GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

Results

Antioxidant effect

The free radical scavenging activity was done to assess the antioxidant capacity of banana sap extract. With the increase in concentration of the banana sap extract, scavenging activity increased significantly. Figure 1 represented antioxidant potential of dichloromethane extract of banana sap. Mean followed by same letter are not significantly different. The highest antioxidant activity was observed to be $54.62 \pm 1.09\%$ at the concentration of 1000 µg/mL. Ascorbic acid (Stock solution: 100 µg/mL; Working volume: 4µL) which was used as a positive control, had a scavenging activity of $89.67 \pm 1.53\%$.

Antimicrobial potential of the banana sap

Banana sap when exposed to air becomes light brown to dark brown due to the presence of tannins. However, during the course of antimicrobial potential evaluation two strategies were adopted: (a) evaluation of banana sap exposed to air (black/dark brown) [(Fig. 2a)] (ii) banana sap remained green as tannins were chelated and was not directly exposed to air [Fig. 2b]. It was observed that the colored sap exhibited a higher minimal inhibitory concentration (MIC) against gram-positive and gram-negative bacteria which was in range of 125 mg/mL to 500 mg/mL while the green sap exhibited a much lower MIC in range of 15.625 mg/mL to 62.5 mg/mL (Table 1). This drastic reduction in the MIC in the tannin chelated (green sap) is probably due to non-interference of tannins. However, both extracts did not exhibit any anti-candidal activity. Amoxicillin is semi-synthetic penicillin that has both gram-positive and gram-negative activity and therefore has been used as a positive control.

Cytotoxic effect in cancer cell lines

DCM extract of banana sap was tested for their effect on MCF-7 (breast cancer cell lines) cell growth based on MTT

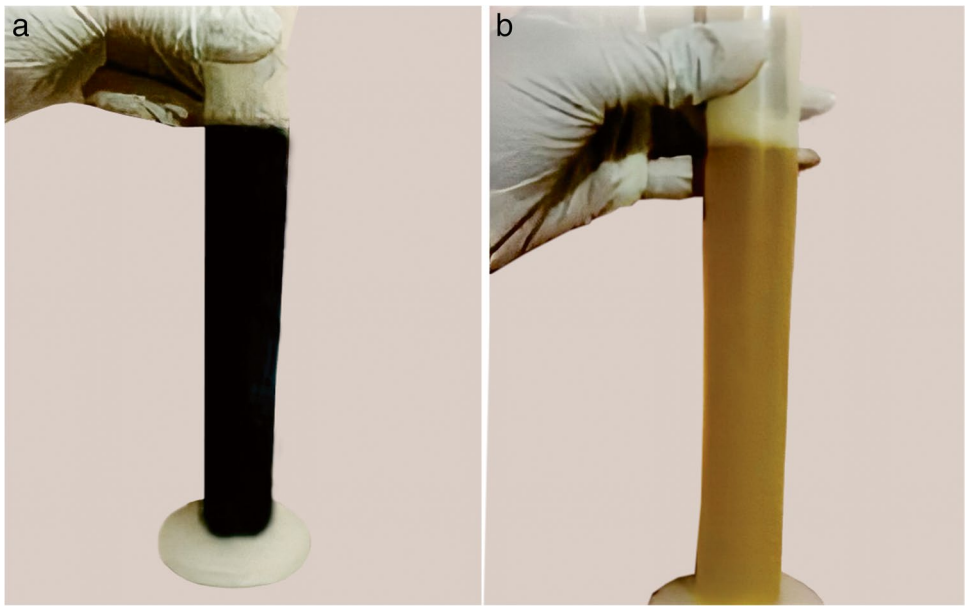


Fig. 2 **a**) Banana sap exposed to air. **b**) Banana sap when not exposed to air

Table 1 Comparative *in vitro* antimicrobial activity as MIC of oxidized and unoxidized Banana sap

Test sample	*MIC values against the test microorganisms (mg/ml)**				
	E. coli	*B. megaterium*	*P. aeruginosa*	*S. aureus*	*C. albicans*
Oxidized banana sap	125	250	500	125	-
Unoxidized banana sap	15.625	62.5	62.5	15.625	-
Positive control (antibacterial) @ 0.1 mg/ml	-	-	-	-	
Positive control (anti-fungal) @ 0.1 mg/ml	-	-	-	-	-

*All values are means of triplicate group

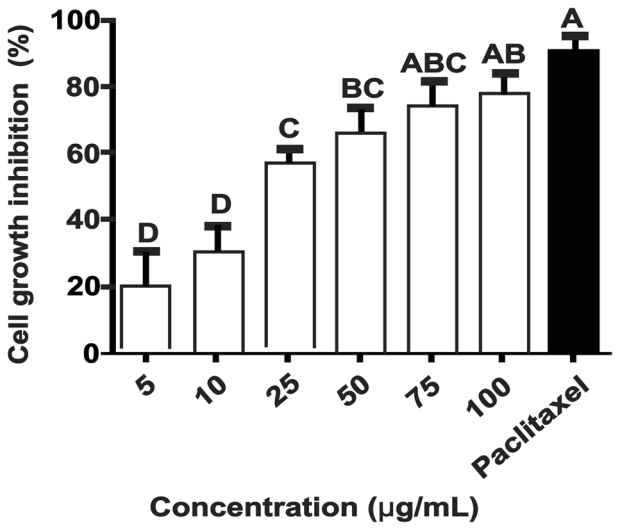


Fig. 3 Cytotoxic effect of dichloromethane extract of banana sap against human breast cancer (MCF-7) cell lines. Mean followed by same letter are not significantly different

assay. It was observed that extracts inhibit the growth of MCF-7 cells representing cytotoxic effect which is found to be significantly increased with concentration. Figure 3 presented cytotoxic effect of dichloromethane extract of banana sap against human breast cancer (MCF-7) cell lines. Mean followed by same letter are not significantly different. The IC_{50} value was calculated and found to be 34.15 ± 8.75 µg/mL. Paclitaxel, an anticancer drug was used as a positive control where the growth inhibition was observed to be $90.81 \pm 4.42\%$.

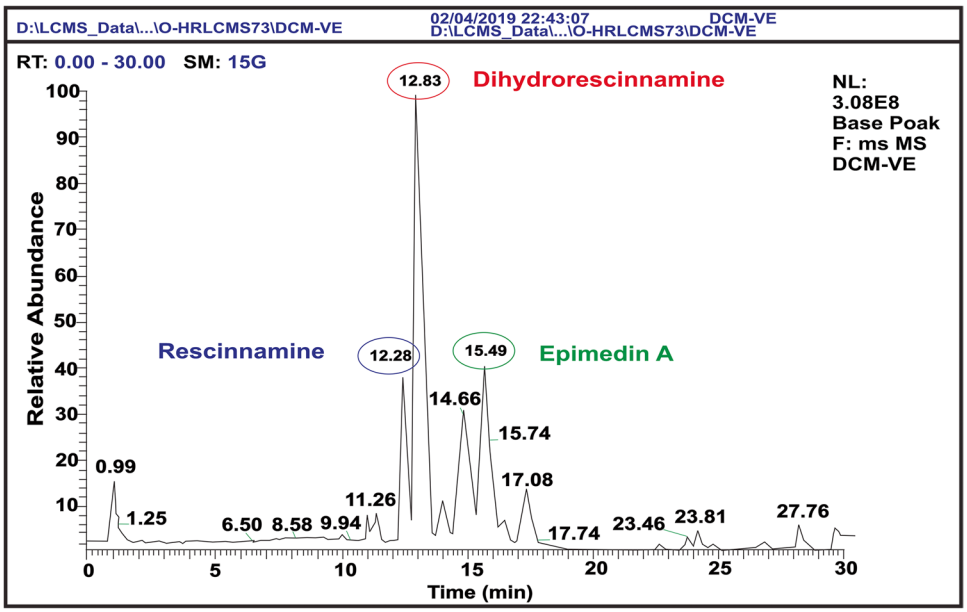
LC–MS analysis

The proposed identifications are based on Orbitrap Liquid Chromatography Mass Spectroscopy chromatograms, mass spectra and a comparison with previous literature and reference data from the m/z library compound database.

Compounds such as rescinnamine, dihydrorescinnamine and epimedin A were predicted based on the mzCloud results of the LC–MS Library (© Reported with Compound Discoverer 2.1). Compounds having RT = 12.28, 12.83 and 15.49 appeared in DCM extracts in major quantities and have been resolved and detected under the column condition. Figure 4 represented spectra of the DCM extract of the banana sap. Figure 5 explained spectra of the RT 12.28 of the DCM extract of the banana sap, displaying fragmentation pattern of compounds in DCM extract of the banana sap. Further, fragmentation pattern of the rescinanmine can be explained by the loss of the $C_{15}H_{16}O_7$ Fig. 5a. Figure 5b explains the fragmentation pattern and products by the loss of $C_{14}H_{24}O_5$ whereas Fig. 5c exhibits the fragmentation pattern by the gain of $C_3H_4N_2$ because of the dimerization of the molecules and loss of the O_3 from the parent compound. Figure 6 presented spectra of the RT 12.83 of the DCM extract of the banana sap, displaying fragmentation pattern of compounds in DCM extract of the banana sap. Moreover, fragmentation pattern of the dihydrorescinnamine Fig. 6a can be explained by the loss of $C_{15}H_{16}O_{7,}$ (b) can be explained by the loss of the $C_{14}H_{22}O_{5,}$ (c) can be explained by the gain of $C_3H_6N_2$ because of the dimerization of the molecules and loss of the O_3 from the parent compound. Figure 7a represented spectra of the RT 15.49 of the DCM extract of the banana sap whereas Fig. 7b explained the fragmentation pattern of compounds at spectra of the RT 15.49 of the DCM extract of the banana sap. Moreover, fragmentation pattern of the Fig. 7b of Epimedin A (a) can be explained by the loss of $C_{21}H_{34}O_{15,}$ (b) can be explained by the loss of $C_{18}H_{32}O_{15,}$ (c) can be explained by the loss of $C_7H_{16}O_{7,}$ (d) can be explained by the loss of the H_4O_2, from the parent compound. There was a difference of the ± 1 in the exact mass and the observed mass which is due to the loss and gain of the H atom.

Discussions

Herbal extracts or plant extracts have been a part of traditional and folklore medicines. Medicinal systems like Ayurveda, Unani, Traditional Chinese medicine rely on the use of plant extracts that have been time tested for their medical potential (Pandey 2021). The interdisciplinary applications of different plant components such as the flower, pulp, stem, and leaves enlist the banana plant among the most beneficial plants (Lopes et al. 2020). The sap from bananas has high



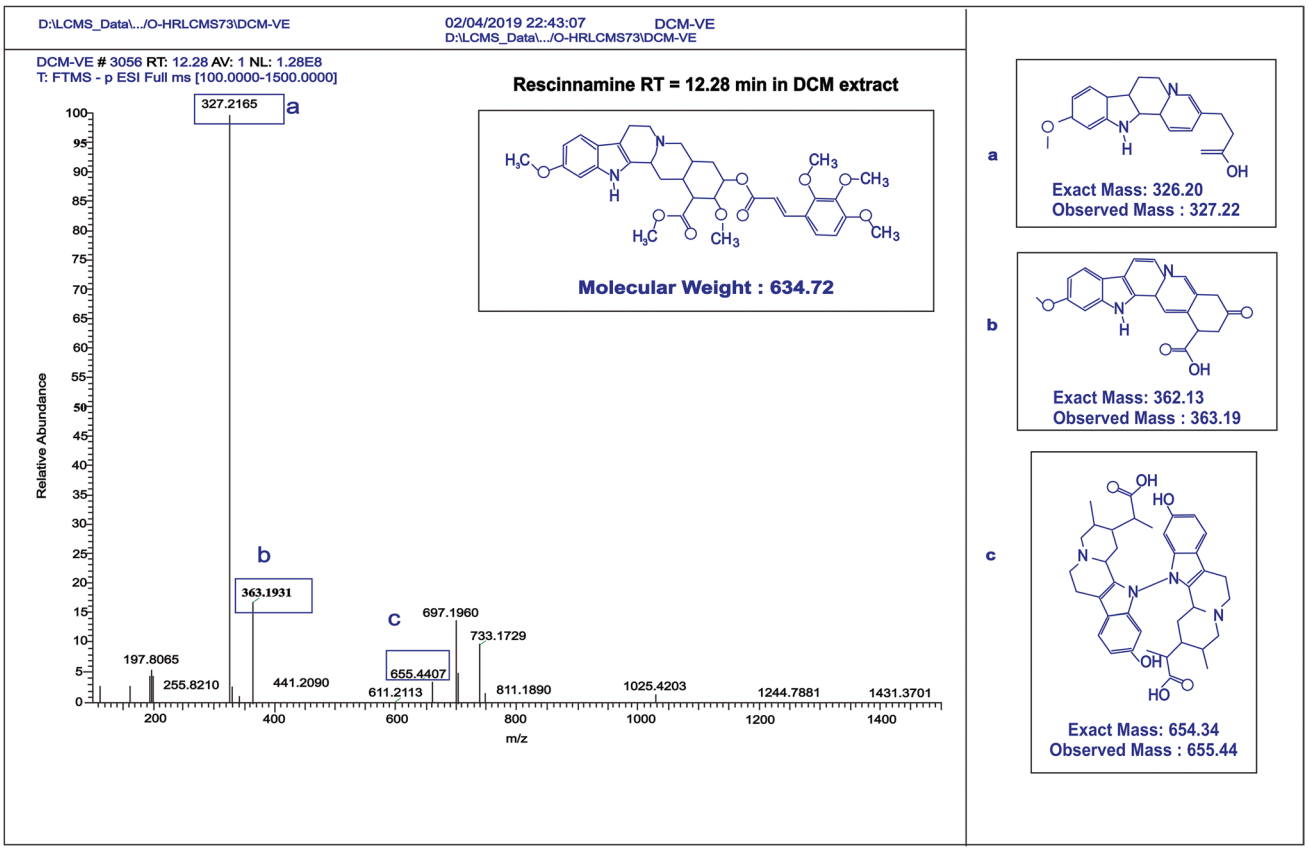


Fig. 4 Spectra of the DCM extract of the banana sap

Fig. 5 Spectra of the RT 12.28 of the DCM extract of the banana sap, displaying fragmentation pattern of compounds in DCM extract of the banana sap

medicinal value and is being used to cure a range of ailments, including bleeding, hysteria, fever, leprosy, digestive problems, epilepsy, hemorrhoids, and insect bites (Kumar et al. 2012 and Gupta et al. 2022). Furthermore, banana sap is an excellent source of the bioactive and antioxidants phytochemicals, such as phenolic and flavonoid compounds

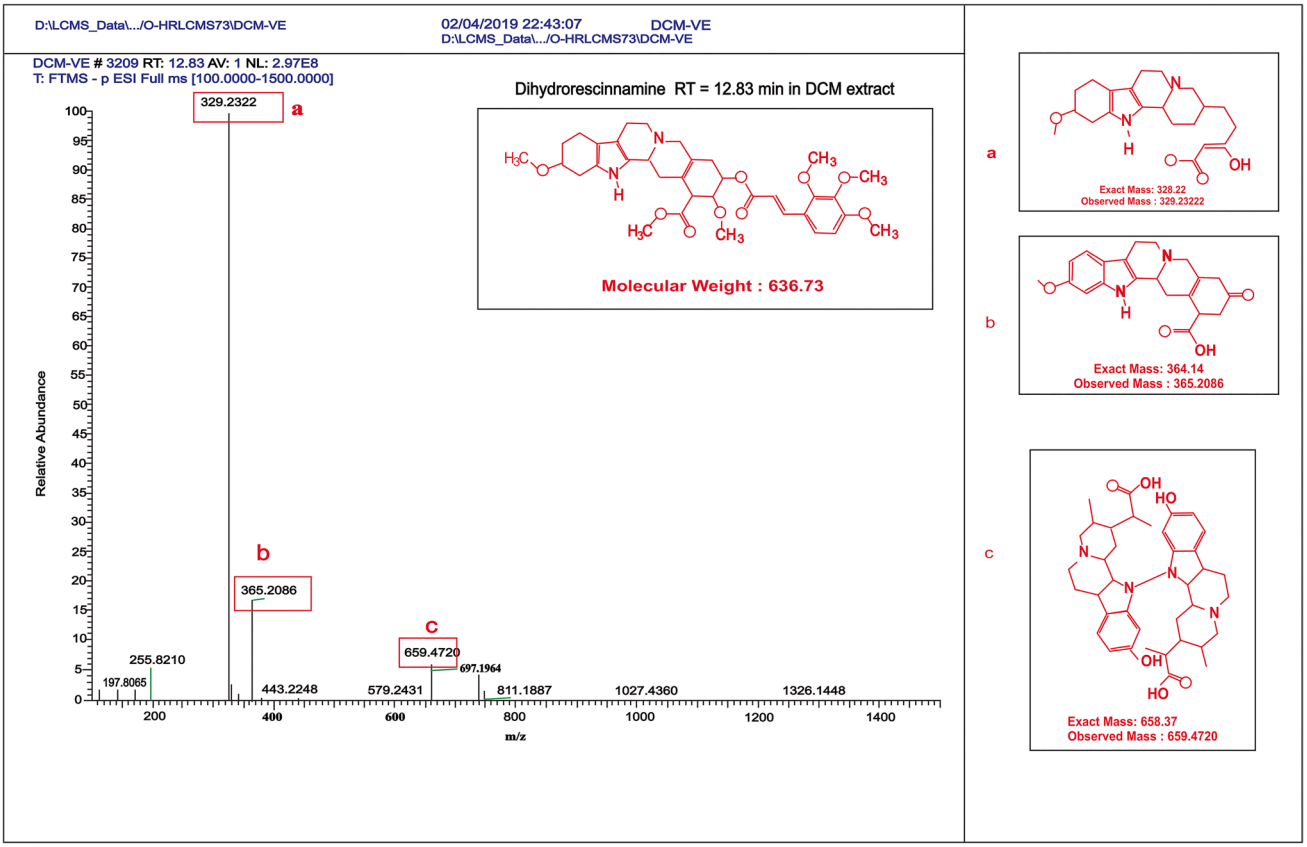


Fig. 6 Spectra of the RT 12.83 of the DCM extract of the banana sap, displaying fragmentation pattern of compounds in DCM extract of the banana sap

such as apigenin glycosides, myricetin glycoside, myricetin-3-o-rutinoside, naringenin glycosides, kaempferol-3-o-rutinoside, quericitin-3-o-rutinoside, dopamine and N-acetyl serotonin (Pothavorn et al. 2010). Hence in this study, we explored the antioxidant, anticancer and antimicrobial potential of banana sap from the pseudo stem. Literature abounds on the antioxidant activity in different parts of the banana plant such as fruit (Alothman et al. 2009), pulp and peel (Sulaiman et al. 2011; Mokbel and Hashinaga 2005; Nagarajaiah and Prakash 2011), leaf (Karuppiah and Mustaffa 2013), pseudo-stem and rhizome (Saravanan and Aradhya 2011; Kumar et al. 2014), and flower (Loganayaki et al. 2010). 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method is the most commonly used followed by ferric reducing antioxidant power (FRAP), total antioxidant activity (TAA) to assess the anti-oxidant potential. Moreover, for the evaluation of antioxidant potential, various extracts were prepared by researchers in water (Alothman et al. 2009; Mokbel and Hashinaga 2005; Nagarajaiah and Prakash 2011), acetone (Loganayaki et al. 2010), hexane (Sulaiman et al. 2011), ethanol (Nagarajaiah and Prakash 2011), and methanol (Kumar et al. 2014). Researchers have demonstrated distinct antioxidant activity of various parts of banana and revealed that solvent-based extracts exhibited higher antioxidant activity compared to aqueous extracts. Pothavorn et al. (2010) reported that the banana sap contains bioactive compounds caffeoylquinic acid or chlorogenic acid which is responsible for its antioxidant activity. In the present study, the antioxidant potential of dichloromethane extract of concentrated banana sap has been evaluated using DPPH assay, however, the anti-oxidant activity was moderate as compared to the ascorbic acid. The antioxidant potential could possibly be attributed to non-polar compounds present in the dichloromethane extract.

Several studies have reported antimicrobial activity from different plant parts of *Musa* spp. The most common microorganisms used for the studies included *B. subtilis, E. coli, S. aureus* and *P. aeruginosa* (Naikwade et al. 2014; Asuquo and Udobi 2016). Although some researchers have also studied *Micrococcus, Klebsiella* and *Salmonella* (Ehiowemwenguan et al. 2014; Kumar et al. 2014), in the test panel of microorganisms. The various fungal species tested include *Candida, Aspergillus, Penicillium, Cryptococcus* and *Trichophyton* (Kumar et al. 2014; Jouneghani et al. 2020). The majority of researchers have studied the antimicrobial activities of banana leaves (Naikwade et al. 2014; Asuquo and Udobi,

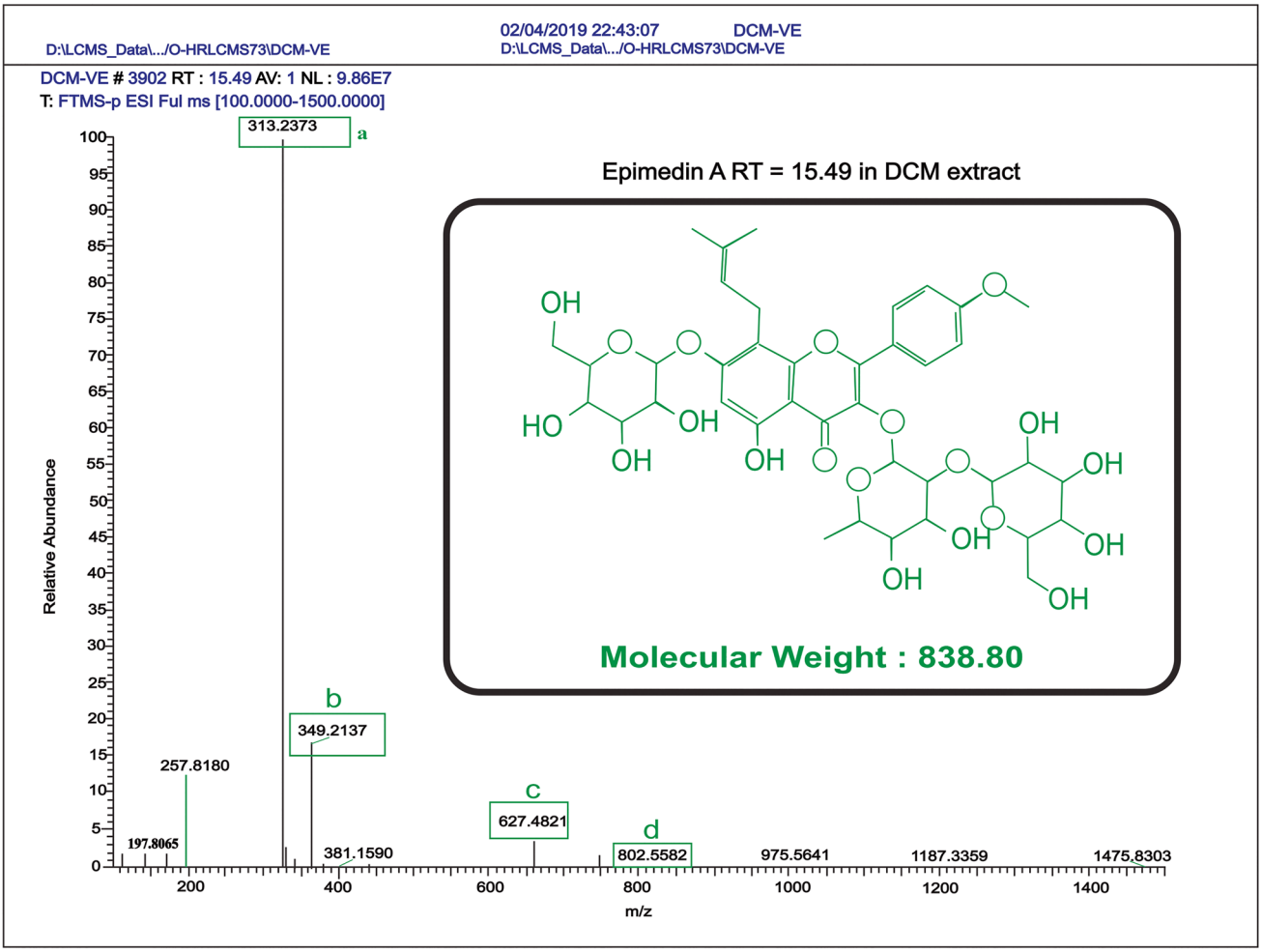


Fig. 7 **a** Spectra of the RT 15.49 of the DCM extract of the banana sap, **b** Spectra of the RT 15.49 of the DCM extract of the banana sap, displaying fragmentation pattern of compounds in DCM extract of the banana sap

2016; Sivasamugham et al. 2021). Apart from leaves, peel (Ehiowemwenguan et al. 2014), inflorescence (Padam et al. 2012) and sap (Kumar et al. 2014) have also been studied for the presence of antimicrobial activities. Both aqueous (Ehiowemwenguan et al. 2014) and organic fractions (Padam et al. 2012; Kumar et al. 2014) of extracts from different parts of banana plants have been tested for their antimicrobial spectrum. It has been observed by researchers in the case of bananas that the aqueous fraction has poor antimicrobial potency as compared to organic extracts. The antimicrobial panel in our studies comprised of both Gram-positive and Gram-negative bacteria. However, our approach was unique in the sense that we evaluated the antimicrobial potential of the oxidized and unoxidized banana sap and proved that the unoxidized banana sap, which was green in colour had potent antimicrobial activity as compared to the oxidized sap in terms of MIC. There was an 8-fold reduction which happened in the unoxidized sap. The oxidation of tannins leads to a black color and becomes unavailable for antibacterial actions. By chelating, its oxidation is stopped and it remains active and induces antibacterial action. To the best of our knowledge the same has never been investigated. Kumar et al. (2014) reported that the banana sap has little antimicrobial activity but no anti-fungal activity was reported since only oxidized sap was evaluated. However, our results of anti-candida activity are in agreement with the results of Kumar et al. (2014).

Moreover, researchers have reported that different parts of banana such as peel, pulp and seed (Li et al. 2013; Zawawy 2015), fruit (Ampasavate et al. 2010; Dahham et al. 2015), flower (Nadumane and Timsina 2014), and banana leaf (Asuquo and Udobi 2016) to possess anti-proliferative activity when tested on various cancerous cell lines such as A549, MCF-7, Hep G2, HT-29, U937, K562, HL60, Molt4, CHO, HUVEC, HCT-116 and swiss albino mice selected from the different cultivars of the Musa. Mostly MCF-7 cell lines have been studied by the researchers. The extracts showed distinct activity with defined IC_{50} values. For instance,

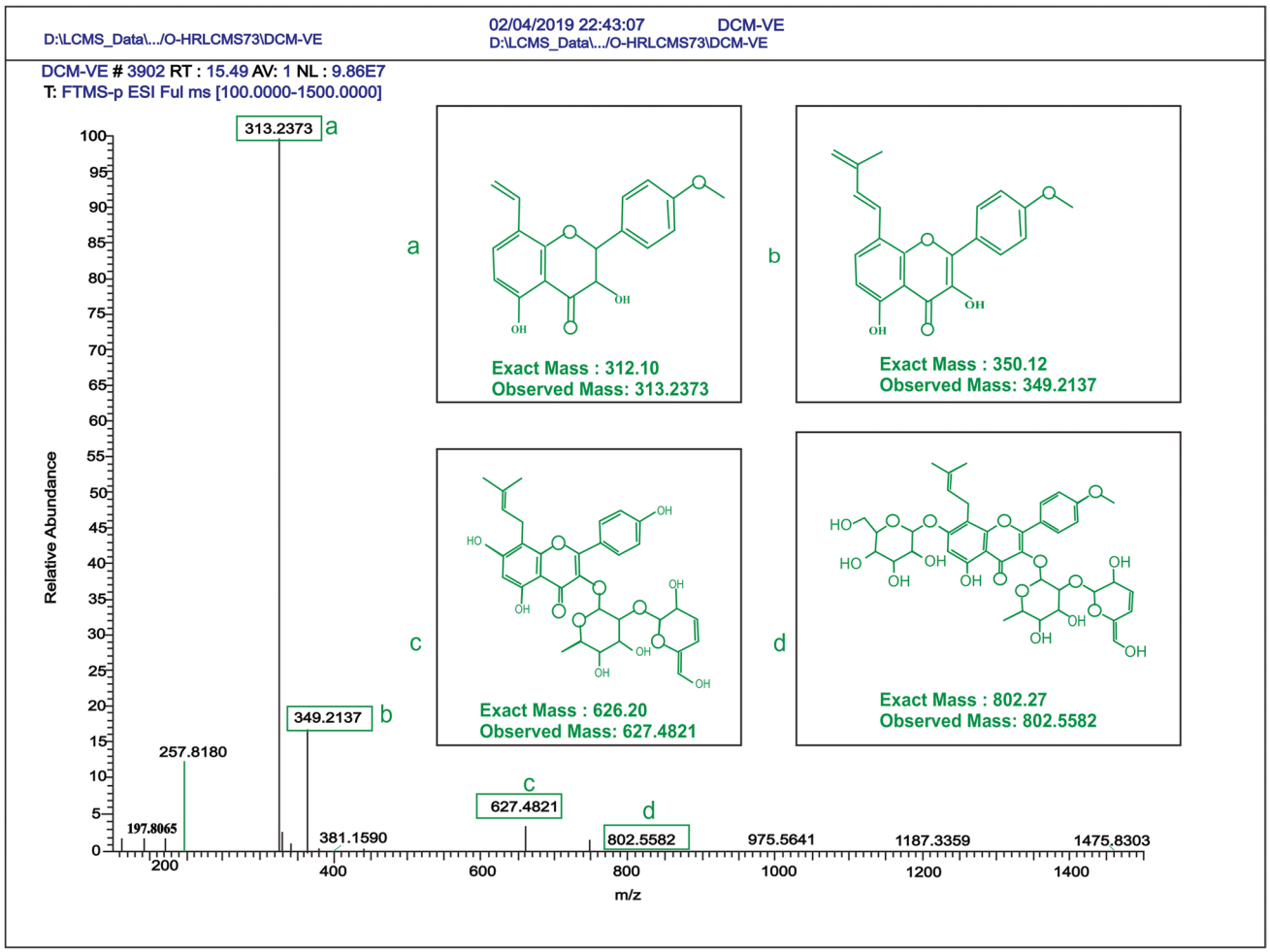


Fig. 7 (continued)

banana flower showed activity with IC_{50} less than 10 µg/mL for HeLa and CHO cell lines (Nadumane and Timsina 2014), banana peel showed upto 33% antiproliferative activity for MCF-7 cell lines (Zawawy 2015). Although banana sap based anti-cancer activity has not been clearly demonstrated in cell lines or animal models but the presence of flavones and flavanol indicates its anti-cancer effect (Pothavorn et al. 2010). However, in our studies we have proved anticancer potential of the dichloromethane extract of the banana sap.

Plants are the key sources of natural phenolics, such as different types of phenolic acids, including a group of hydroxybenzoic acids (Sarker and Oba 2020a) and hydroxycinnamic acids (Sarker and Oba 2018), flavonoids, including flavonols (Sarker and Oba 2019), flavones (Sarker and Oba 2020b) flavanols (Sarker and Oba 2020c) flavanones (Sarker et al. 2020) isoflavones, anthocyanins, chalcones, and nonflavonoids, including tannins, lignans, and stilbenes (González-Sarrías et al. 2020). Major phytochemicals compounds reported from the banana sap can be grouped as alkaloids, phenolics, saponins, lignins, coumarins and cardiac glycosides (Onyema et al. 2016; Pothavorn et al. 2010). Furthermore, phytochemical analysis of banana sap varieties such as *Musa balbisiana, Musa laterita, Musa ornate and Musa acuminate* shows that sap is rich in flavones and flavanols (Pothavorn et al. 2010). HPLC based identification has shown that apigenin glycosides (which inhibits many types of cancer cell lines by promotion of cell cycle arrest and apoptosis), naringenin glycosides are very abundant in sap. Flavanols such as myrecetin glycoside, querecetin glycosides and kaempferol are also detected in high amounts in sap. Other than flavanols and flavones components such as dopamine and N-acetyl serotonin are also detected in sap. Flavanols and flavones are known anti-cancer compounds (Pothavorn et al. 2010). Nguyen et al. (2017) reported several antidiabetic compounds such as lupeol, ferulic acid, vanillic acid, trans-cinnamic acid, p-hydroxybenzoic acid, p-coumaric acid, rutin, catechin/epicatechin, chlorogenic acid, gallic acid, caffeic acid and nicotiflorin from the banana stem juice of *M. paradisiaca.* In our study LC–MS data detected the presence of alkaloid

and flavonoids as major compounds such as rescinnamine derivative, dihydrorescinnamine and epimedin A in DCM extract of banana sap.

The presence of rescinnamine has not been reported from any banana plant or cultivar previously. Alkaloids are largest group of secondary chemical constituents comprising nitrogen-containing naturally occurring chemicals that have been shown to have antibacterial effects owing to their tendency to intercalate with genetic material of the microorganisms (Ramu et al. 2015; Ogbonna et al. 2016). Furthermore, alkaloids have analgesic, anti-spasmodic and bactericidal effects and this is the basis for their use as basic medicinal agents (Okwu 2004). Rescinnamine is an anti-hypertensive drug which inhibits coronavirus binding to a receptor in the cell surface of human cell (Wu et al. 2020). Based on this, we can say that observed antimicrobial activity of banana sap in our study is due to the presence of the alkaloid compounds such as rescinnamine, dihydrorescinnamine. Likewise, epimedins have not been reported till date in banana. In fact, there are no reports on presence of glycosylated flavonoids in banana. Moreover, flavonoids are powerful antioxidants with anti-inflammatory, anti-neoplastic, anti-cancer, anti-allergic, anti-viral and hepatoprotective properties (Lewis et al. 1999; Xi et al. 2014; Xie et al. 2015; Ogbonna et al. 2016). According to data from *in vitro* and *in vivo* investigations, epimedin compounds have strong anticancer effect against a wide spectrum of cancer cells via a variety of pathways including apoptosis, cell cycle regulation, anti-angiogenesis, anti-metastasis, and immunological modulation (Tan et al. 2016; Lone et al. 2018). In another investigation on *E. koreanum*, all four Epimedium species markers, epimedin A, epimedin B, epimedin C and icariin showed high anticancer potential (Yasukawa et al. 2016). As a result, Epimedium herbs might be beneficial in cancer prevention. Our study reported the epimedin A in banana sap and also showed the presence of anti-oxidant, anti-cancer, antimicrobial activities in the banana sap. Therefore, our study is in agreement with the findings of Yasukawa et al. (2016).

Conclusions

Our present study has provided an insight on the potential of banana pseudo-stem sap as a good source of bioactive compounds which may possess a host of pharmacologically relevant properties beyond antioxidant, antimicrobial and anticancer activities. These bioactive properties may be associated with the presence of rescinnamine derivative, dihydrorescinnamine and epimedin A. Our study is a primer of opening up metabolomic studies under non-oxidized state of banana sap with a possibility of their applications in the herbal cosmeceutical and nutraceutical preparations thereby valorising the banana sap which is currently attributed as a waste product.

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Author contributions GG collected the plant material, prepared extracts; SS, MBW and MSR supervised the study; GG performed the experiments, made the statistical analysis of the data and prepared the manuscript. All authors revised the manuscript.

Declarations

Conflict of interest: The authors declare that they have no conflict of interest.

References

Alothman M, Bhat R, Karim AA (2009) Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chem 115:785–788. https://doi.org/10.1016/j.foodchem.2008.12.005

Ampasavate C, Okonogi S, Anuchapreeda S (2010) Cytotoxicity of extracts from fruit plants against leukemic cell lines. Afr J Pharm Pharmacol 4:013–021. https://doi.org/10.5897/AJPP.9000092

Asuquo EG, Udobi CE (2016) Antibacterial and toxicity studies of the ethanol extract of Musa paradisiaca leaf. Cogent Biol 2:1219248. https://doi.org/10.1080/23312025.2016.1219248

Borges MH, Alves DLF, Raslan DS, Piló-Veloso D, Rodrigues VM, Homsi-Brandeburgo MI, De Lima ME (2005) Neutralizing properties of Musa paradisiaca L. (Musaceae) juice on phospholipase A2, myotoxic, hemorrhagic and lethal activities of crotalidae venoms. J Ethnopharmacol 98:21–29. https://doi.org/10.1016/j.jep.2004.12.014

Budi HS, Juliastuti WS, Christy BR (2020) Antimicrobial Activity of Musa paradisiaca var. sapientum on Enterococcus faecalis Viability. Mal J Med Health Sci 16:17–21

Dahham SS, Mohamad TA, Tabana YM, Majid AMSA (2015) Antioxidant activities and anticancer screening of extracts from banana fruit (Musa sapientum). Acad J Cancer Res 8:28–34. https://doi.org/10.5829/idosi.ajcr.2015.8.2.95162

Dikshit P, Tyagi MK, Shukla K, Gambhir JK, Shukla R (2016) Antihypercholesterolemic and antioxidant effect of sterol rich methanol extract of stem of Musa sapientum (banana) in cholesterol fed wistar rats. J Food Sci Technol 53:1690–1697. https://doi.org/10.1007/s13197-015-2096-5

Ehiowemwenguan G, Emoghene AO, Inetianbor JE (2014) Antibacterial and phytochemical analysis of Banana fruit peel. Iosr J Pharm 4:18–25

El Zawawy NA (2015) Antioxidant, antitumor, antimicrobial studies and quantitative phytochemical estimation of ethanolic extracts of selected fruit peels. Int J Curr Microbiol Appl Sci 4:298–309

González-Sarrías A, Tomás-Barberán FA, García-Villalba R (2020) Structural diversity of polyphenols and distribution in foods. In Dietary Polyphenols: Their Metabolism and Health Effects, Wiley, pp 1–29. https://doi.org/10.1002/9781119563754.ch1

Gupta G, Baranwal M, Saxena S, Reddy MS (2019) Utilization of banana stem juice as a feedstock material for bioethanol

production. CLEAN–Soil Air Water 47:1–5. https://doi.org/10.1002/clen.201900047
Gupta G, Baranwal M, Saxena S, Reddy MS (2022) Utilization of banana waste as a resource material for biofuels and other value-added products. Biomass Conver Bioref: 1-20. https://doi.org/10.1007/s13399-022-02306-6
Jorgensen JH, Turnidge JD, Washington JA, Murray EJ, Pfaller MA, Tenover FC, Yolken RH (1999) Manual of clinical microbiology 7thed, American Society for Microbiology. ASM Press, Washington DC, pp 1526–1543
Jouneghani RS, Castro AHF, Panda SK, Swennen R, Luyten W (2020) Antimicrobial activity of selected banana cultivars against important human pathogens, including candida biofilm. Foods 9:435. https://doi.org/10.3390/foods9040435
Kar GA, Rout SK, Mishra D (2019) Evaluation of antidepressant Activity of methanolic Extract of Musa x paradisiaca Linn using animal models. Am J Pharm Tech Res 9:315–320
Karuppiah P, Mustaffa M (2013) Antibacterial and antioxidant activities of Musa sp. Leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pac J Trop Biomed 3:737–742. https://doi.org/10.1016/S2221-1691(13)60148-3
Khamboonruang P, Anuntasethakul T, Navephap S, LeGrand SM (2015) Evaluation of anti-gastric ulcer activity of ethanolic extract from pseudostem of Musa× paradisiaca in experimental animals. Thai J Sci Technol 4:310–320. https://doi.org/10.14456/tjst.2015.1
Kumar KS, Bhowmik D, Duraivel S, Umadevi M (2012) Traditional and medicinal uses of banana. J Pharmacogn Phytochem 1:51–63
Kumar PR, Srivastava S, Singh KK, Mathad C, Thin PS (2014) Study of Antioxidant and Antimicrobial Properties, Phytochemical screening and analysis of Sap Extracted from Banana (Musa acuminata) pseudostem. Int J Adv Biotechnol Res 5:649–658
Lewis DA, Fields WN, Shaw GP (1999) A natural flavonoid present in unripe plantain banana pulp (Musa sapientum L. var. paradisiaca) protects the gastric mucosa from aspirin-induced erosions. J Ethnopharmacol 65:283–288. https://doi.org/10.1016/S0378-8741(99)00005-7
Li F, Li S, Li HB, Deng GF, Ling WH, Wu S, Xu XR, Chen F (2013) Antiproliferative activity of peels, pulps and seeds of 61 fruits. J Funct Foods 5:1298–1309. https://doi.org/10.1016/j.jff.2013.04.016
Loganayaki N, Rajendrakumaran D, Manian S (2010) Antioxidant capacity and phenolic content of different solvent extracts from banana (Musa paradisiaca) and mustai (Rivea hypocrateriformis). Food Sci Biotechnol 19:1251–1258. https://doi.org/10.1007/s10068-010-0179-7
Lohia N, Baranwal M (2017) Immune responses to highly conserved influenza A virus matrix 1 peptides. Microbiolo Immunol 61:225–231. https://doi.org/10.1111/1348-0421.12485
Lone SA, Gupta AP, Manzoor MM, Goyal P, Hassan QP, Gupta S (2018) Epimedium elatum (morr & decne): a therapeutic medicinal plant from northwestern himalayas of India. Plant and Human Health, vol 1. Springer, Cham, pp 619–656
Lopes S, Borges CV, de Sousa Cardoso SM, de Almeida Pereira da Rocha MF, Maraschin M (2020) Banana (Musa spp.) as a Source of Bioactive Compounds for Health Promotion. Handbook of Banana Production, Postharvest Science, Processing Technology and Nutrition, Wiley, pp 227–244
Majhi S, Das D (2021) Chemical derivatization of natural products: semisynthesis and pharmacological aspects-A decade update. Tetrahedron 78:131801. https://doi.org/10.1016/j.tet.2020.131801
Mokbel MS, Hashinaga F (2005) Antibacterial and antioxidant activities of banana (Musa, AAA cv. Cavendish) fruits peel. Am J Biochem Biotechnol 1:125–131. https://doi.org/10.3844/ajbbsp.2005.125.131
Nadumane VK, Timsina B (2014) Anticancer potential of banana flower extract: An in vitro study. Bangladesh J Pharmacol 9:628–635. https://doi.org/10.3329/bjp.v9i4.20610
Nagarajaiah SB, Prakash J (2011) Chemical composition and antioxidant potential of peels from three varieties of banana. As J Food Ag-Ind 4:31–46
Naikwade PV, Gaurav S, Sharayu D, Kailas J (2014) Evaluation of antibacterial properties of Musa paradisiaca L. Leaves. In: Proceeding of the national conference on conservation of natural resources & biodiversity for sustainable development, pp 80–84
Nguyen D, Novakova A, Spurna K (2017) Antidiabetic compounds in stem juice from banana. Czech J Food Sci 35:407–413. https://doi.org/10.17221/172/2017-CJFS
Ogbonna OA, Izundu AI, Okoye NH, Ikeyi AP (2016) Phytochemical compositions of fruits of three Musa species at three stages of development. IOSR J Pharm Biol Sci 11:48–59. https://doi.org/10.9790/3008-1103044859
Okwu DE (2004) Phytochemical and vitamin content of indigenous spices of South Eastern Nigeria. J Sustain Agric Environ 6:30–34
Onyema CT, Ofor CE, Okudo VC, Ogbuagu AS (2016) Phytochemical and antimicrobial analysis of banana pseudo stem (Musa acuminata). J Pharm Res Int 10:1–9. https://doi.org/10.9734/BJPR/2016/22593
Padam BS, Tin HS, Chye FY, Abdullah MI (2012) Antibacterial and Antioxidative Activities of the Various Solvent Extracts of Banana (Musa paradisiaca cv. Mysore) Inflorescences. J Biol Sci 12:62–73
Pandey A (2021) A Glimpse into the Indian Traditional Medicine with Special Reference to Use of Hemidesmus indicus in Southern India: A review. Inter J Theor Appl Sci 13:73–79
Pothavorn P, Kitdamrongsont K, Swangpol S, Wongniam S, Atawongsa K, Svasti J, Somana J (2010) Sap phytochemical compositions of some bananas in Thailand. J Agric Food Chem 58:8782–8787. https://doi.org/10.1021/jf101220k
Ramu R, Shirahatti P, Zameer F, Lakkapa DB, Nagendra Prasad MN (2015) Evaluation of Banana (Musa sp. var. Nanjangud Rasa bale) flower and pseudostem extracts on antimicrobial, cytotoxicity and thrombolytic activities. Int J Pharm Pharm Sci 7:136–140
Saravanan K, Aradhya SM (2011) Potential nutraceutical food beverage with antioxidant properties from banana plant bio-waste (pseudostem and rhizome). Food Funct 2:603–610. https://doi.org/10.1039/C1FO10071H
Sarker U, Hossain M, Iqbal M, Oba S (2020) Bioactive components and radical scavenging activity in selected advance lines of salt-tolerant vegetable amaranth. Front Nutr 7:587257. https://doi.org/10.3389/fnut.2020.587257
Sarker U, Oba S (2018) Augmentation of leaf color parameters, pigments, vitamins, phenolic acids, flavonoids and antioxidant activity in selected Amaranthus tricolor under salinity stress. Sci Rep 8:10–1038. https://doi.org/10.1038/s41598-018-30897-6
Sarker U, Oba S (2019) Antioxidant constituents of three selected red and green color Amaranthus leafy vegetable. Sci Rep 9:1–11. https://doi.org/10.1038/s41598-019-52033-8
Sarker U, Oba S (2020a) Phenolic profiles and antioxidant activities in selected drought-tolerant leafy vegetable amaranth. Sci Rep 10:18287. https://doi.org/10.1038/s41598-020-71727-y
Sarker U, Oba S (2020b) Polyphenol and flavonoid profiles and radical scavenging activity in leafy vegetable Amaranthus gangeticus. BMC Plant Biol 20:1–12. https://doi.org/10.1186/s12870-020-02700-0
Sarker U, Oba S (2020c) Nutraceuticals, phytochemicals, and radical quenching ability of selected drought-tolerant advance lines of vegetable amaranth. BMC Plant Biol 20:564. https://doi.org/10.1186/s12870-020-02780-y
Sharma V, Lohia N, Handa V, Baranwal M (2017) Amomum subulatum seed extract exhibit antioxidant, cytotoxic and immune-suppressive effect. Indian J Biochem Biophys 54:135–139

Sheng Z, Dai H, Pan S, Ai B, Zheng L, Zheng X, Prinyawiwatkul W, Xu Z (2017) Phytosterols in banana (Musa spp.) flower inhibit α-glucosidase and α-amylase hydrolysations and glycation reaction. Int J Food Sci Technol 52:171–179. https://doi.org/10.1111/ijfs.13263

Sivasamugham LA, Nimalan V, Subramaniam G (2021) Antibacterial effects of Musa sp. ethanolic leaf extracts against methicillin-resistant and susceptible Staphylococcus aureus. South African J Chem Eng 35:107–110

Sulaiman SF, Yusoff NAM, Eldeen IM, Seow EM, Sajak AAB, Ooi KL (2011) Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (Musa sp.). J Food Compos Anal 24:1–10. https://doi.org/10.1016/j.jfca.2010.04.005

Tan HL, Chan KG, Pusparajah P, Saokaew S, Duangjai A, Lee LH, Goh BH (2016) Anti-cancer properties of the naturally occurring aphrodisiacs: icariin and its derivatives. Front Pharmacol 7:191. https://doi.org/10.3389/fphar.2016.00191

The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 8.0, 2018. http://www.eucast.org

Vasundhara M, Baranwal M, Sivaramaiah N, Kumar A (2017) Isolation and characterization of trichalasin-producing endophytic fungus from Taxus baccata. Ann Microbiol 67:255–261. https://doi.org/10.1007/s13213-017-1256-4

Wu C, Chen X, Cai Y, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, Zhang Y, Song J (2020) Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med 180:934–943. https://doi.org/10.1001/jamainternmed.2020.0994

Xi W, Fang B, Zhao Q, Jiao B, Zhou Z (2014) Flavonoid composition and antioxidant activities of Chinese local pummelo (Citrusgrandis Osbeck.) varieties. Food Chem 161:230–238. https://doi.org/10.1016/j.foodchem.2014.04.001

Xie Y, Zheng Y, Dai X, Wang Q, Cao J, Xiao J (2015) Seasonal dynamics of total flavonoids contents and antioxidant activity of Dryopteriserythrosora. Food Chem 186:113–118. https://doi.org/10.1016/j.foodchem.2014.05.024

Yakubu MT, Nurudeen QO, Salimon SS, Yakubu MO, Jimoh RO, Nafiu MO, Akanji MA, Oladiji AT, Williams FE (2015) Antidiarrhoeal activity of Musa paradisiaca Sap in Wistar rats. Evid Based Complement Altern Med. https://doi.org/10.1155/2015/683726

Yasukawa K, Ko SK, Whang WK (2016) Inhibitory effects of the aerial parts of Epimedium koreanumon TPA-induced inflammation and tumour promotion in two-stage carcinogenesis in mouse skin. J Pharm Nutr Sci 6:35–42