



STUDY OF CYTOTOXIC AND ANTIBACTERIAL POTENTIAL OF VARIOUS VARIETIES AND POLARITIES OF EXTRACTS OF UNRIPE BANANAS

Latifa Rashid Abdullah Al-Mqbali¹, Mohammad Amzad Hossain¹, S. Al Touby²

¹School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, P.O. Box 33, Postal Code 616, Nizwa, Sultanate of Oman

²School of Nursing, College of Pharmacy and Nursing, University of Nizwa, P.O. Box 33, Postal Code 616, Nizwa, Sultanate of Oman

✉ amzad@unizwa.edu.om

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ABSTRACT

Different ethnic communities traditionally use herbal medicines to treat different curable and incurable diseases. The main purpose of this research was to assess the antibacterial and cytotoxic potential of different polarities extracts of unripe bananas collected from Omani farmers. The antibacterial and cytotoxic potential of the prepared extracts were assessed against the gram + and - antibacterial strains and brine shrimp lethality (BSL) bioassays. All prepared extracts at dissimilar concentrations exhibited acceptable antibacterial potential against two Gram-positive and two Gram-negative bacteria strains with a diameter of inhibition range of 0-11.5 mm. The maximum antibacterial activity among the plant extracts was obtained from hexane extract against *E. coli*. The cytotoxic results exposed that all polarities unripe banana extracts at each concentrations lethaled the shrimp larvae at all applied concentrations. Among the six polarities extracts of unripe banana from Sohar and Dhofar, the butanol and hexane extracts exhibited significant cytotoxic potential with an LC₅₀ value of 20.12±0.10; 26.19±0.57 and 22.39±0.11; 27.88±0.17 µg/ml. The lesser potential found in ethyl acetate extract among the six extracts with an LC₅₀ value of 36.68±0.22 and 49.32±0.16 µg/ml. The results displayed that non-polar unripe banana extract has substantial antibacterial and cytotoxic potential. Therefore, the significant extract from the unripe banana can be used further for isolation of biologically active ingredients against a number of ailments.

1. Introduction

Plants are considered as an alternative source of antibiotics or chemotherapy agents (Marjorie, 1999). They play a significant role in drug discovery due to their medicinal values to prevent diseases (Marjorie, 1999; Adinarayana & Babu, 2011). Some of them were already detected and characterized to be the best source of antibacterial products (Cintia et al., 2013). Among them, many species all over the world

still remain unexplored (Cintia et al., 2013). In most of the cases, the less developed countries rely only on plant based medicine, which is available locally as a primary form of the health care system. All plant based medicines spread quickly in the form of herbal safe medicine in developed countries due to their active ingredients (Ahmed et al., 2016). Plants, fruits, vegetables and their prepared extracts are more

active than chemically prepared medicines with limited side effects (Al-Matani et al., 2015). Several evidences suggested that increased consumption of fresh unripe and ripe fruits have a lower risk of cancer (Chen et al., 2004). Several chemical ingredients like phenol derivatives, heterocyclic nitrogenous compounds and cardiac glycoside derivatives are vital part any diet (Latifa & Hossain, 2019). In addition, the mentioned groups of ingredients show a variety of pharmacological activities. The pharmacological and biological functions in the human body are directly related to the interference in different stages of malignancy (Debabandya et al., 2010). Bananas are the most popular fruits available everywhere based on nutritive values. Numerous varieties and species are cultivated in different countries including Oman. Banana is a rich nutritional source belonging to Musaceae family. It is originated in Southeast Asia and the South Pacific around 8000 to 5000 BC. From the literature, this fruit is one of the oldest cultivated crops (Khair, 2013). Long-time, most tropical countries commercially produce the fruit on a regular basis. Banana is used as an important source of instant energy as well as an important element of a healthy diet all over the world (Khair, 2013). It is the fourth cultivated agricultural crops and most priority traded crops in the world market (Khair, 2013).

The banana plant is about 12 to 15 feet in height and the leaves are spirally arranged. The whole plant:stems, leaves and fruit have significant medicinal values. There are numerous literature data showing that banana extracts are a rich source of different acids such as fatty acids, linoleic, linolenic, mannose and oleic acids and other active compounds including sterols and steryl esters, fructose, xylose, galactose and glucose (Liu, 2004; Mallikarjuna & Jyothirmay, 2011; Natcharee & Sudip 2011). Recently, some other rare ingredients have been found in bananas, which are responsible for biological activities (Nessma, 2015; Oliveira et al., 2008). Currently, researchers have found that chemical ingredients existing as a mixture in the extracts

of banana are pharmacologically more significant than the individual ingredients to protect malignance due to its synergistic effect (Pereira & Maraschin, 2015). Traditionally, the stem juice of the plant is used to treat acute diarrhea and dysentery, epilepsy and hysteria (Oliveira et al., 2008). The flower extract of the plant is used to treat acute bronchitis, dysentery and ulcers (Liu, 2004; Mallikarjuna & Jyothirmay, 2011; Natcharee & Sudip 2011) and the syrup of cooked flowers is used for acute dysentery and diarrhea, fever, diabetes, epilepsy, leprosy, hysteria and hemorrhages (Liu, 2004; Mallikarjuna & Jyothirmay, 2011; Natcharee & Sudip 2011). The paste made of leaves is used for the treatment of burns, various infections, dysentery, diarrhea and ulcers (Liu, 2004; Mallikarjuna & Jyothirmay, 2011; Natcharee & Sudip 2011). In addition, the paste made of roots is used for the treatment of dysentery and other chronic diseases (Liu, 2004; Mallikarjuna & Jyothirmay, 2011; Natcharee & Sudip 2011). In Oman, local communities use different herbal preparations from bananas for the treatment of indigestion, constipation and diarrhea. Still, there is no preliminary work conducted on the unripe local species. Therefore, it is necessary to determine the antibacterial and cytotoxic activities of unripe bananas that are available in Oman. In this regard, this study was to prepare the crude extracts from different varieties and determine the antibacterial and cytotoxic activities of the prepared extracts.

2. Materials and methods

2.1. Materials

All solvents and chemicals such as hexane (purity 98.3%), ethyl acetate (purity 96.9%), butanol (purity 99.1%), methanol (purity 95.55%), chloroform (purity 89.45%) and acetone (purity 97.01%) were bought from Sigma Aldrich Company, Germany. The reagents and chemicals for this experiment, especially NaCl and Na₂SO₄ were bought from BDH, UK. The broad spectrum antibiotic levofloxacin as well as dimethyl sulphoxide (DMSO) were bought from E. Merck, Germany.

In this experiment, all the glassware used were from Brosil, India.

2.2. Microorganisms

Two gram-positive antibacterial strains such as *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pneumoniae* (*S. pneumoniae*), and two gram-negative *Escherichia coli* (*E. coli*) and *Haemophilus influenza* (*H. influenza*) used in this experiment were obtained from local Hospital, Nizwa, Oman in January 2017.

2.3. Sample collection

The unripe bananas were collected from gardens in Dhofar and Sohar regions, Oman. The samples were collected from farmers in December 31, 2016. The samples were carried home for the necessary steps of extraction. The identification of unripe bananas was done by the local people and match with the website (Latifa & Hossain, 2019).

2.4. Preparation of extracts

The selected unripe banana samples of approximately the same size were cleaned by water and sliced for drying. The sliced fruit was dried under the sun for 5 days. During the drying, the samples were turned over every day to avoid antibacterial contamination. After completely dried, the samples were ground into a coarse powder. The coarse powder sample (350 gm) was packed in white cloth and kept into the two-liter capacity amber beaker for extraction. Methanol (1 L) was added to the beaker and left it for 48 hours. After 48 hours, the coarse powder samples were filtered by vacuum filter and the filtrate was evaporated at 22°C. The methanol free extract (20 gm) was liquefied in water and fractionation with different polarity of solvents starting from non-polar hexane (Rao et al., 2012) to give the corresponding extracts. The extraction process was repeated twice and the mother solvent was evaporated from each extract by using the same way and all dried organic extracts were used to assess the antibacterial and cytotoxic potential.

2.5. Antibacterial potential

The six extracts with different polarity were used to determine their antibacterial potential against two Gram positive bacteria: *S. aureus* and *S. pneumoniae* and two Gram negative bacteria: *E. coli* and *H. influenza* by using modified gel diffusion assay (Rao et al., 2016; Reinisalo et al., 2015). Both antibiotic levofloxacin and DMSO solvents were used as controls. The concentration of levofloxacin was 0.5 mg/ml in DMSO. Filter paper (Whatmann) as disc (diameter 6 mm) was used in the present study, which was prepared by a punch machine. The six polarities extracts at four concentrations such as 2, 1, 0.5 and 0.25 mg/ml were used to assess the antibacterial potential. The discs were initially disinfected and soaked for 30 minutes with each concentration of extract and apply on the inoculated agar plates. All plates with samples were hatched at 37°C for 24 hours (Latifa & Hossain, 2019). The zone of inhibition as diameter was measured by scale against the tested antibacterial strains. It was repeated three times for average zone of inhibition. The antibacterial potential was evaluated of each concentration of all extracts by using the reputable formula.

Antibacterial potential = Diameter zone of inhibition of the sample/Diameter zone of inhibition of the standard.

2.6. Cytotoxic potential

The cytotoxic potential of the extracts was analyzed by the BSL method reported (Latifa & Hossain, 2019). The artemia cysts were incubated in a duo compartment plastic container containing artificial seawater (250 ml) for 24 hours. After 24 hours hatching, the live nauplii were transferred from one compartment to the opposite compartment. In this present experiment, those live nauplii were used to determine cytotoxic potential. six extracts at various concentrations (10, 100, 250, 500 µg/ml) were prepared by using H₂O. Each concentration of each solution, 100 µl samples were placed in the working tube containing 4.9 ml of artificial seawater with 10 live nauplii. After incubation, the surviving nauplii were calculated using a

powerful glass. The percentage of lethality of brine shrimps as well as IC_{50} was calculated for each test sample by using Microsoft Excel.

3. Results and discussions

The banana samples were collected from Sohar and Dhofar, Oman where bananas are cultivated on a large scale. Afterwards, the unripe samples were sliced and kept under the sun for 5 days. The dried banana samples were ready for grinding.

3.1. Preparation of different extracts

The powder samples were used to prepare various polarity extracts by using a maceration method for several days. The prepared extracts and their mass were presented in Table 1.

Table 1. Yield of crude extracts of Sohar and Dhofar's unripe banana

Extracts	Yield of extracts (gm)		Percentage yield of extracts (%)	
	Sohar	Dhofar	Sohar	Dhofar
Hexane	5.28	4.35	26.40	21.75
Ethyl acetate	4.22	3.82	21.10	19.10
Chloroform	3.19	5.13	15.95	25.65
Butanol	2.97	3.11	18.85	15.55
Methanol	38.7	13.91	11.05	6.98
Water	3.82	3.85	19.10	19.25

The values are means \pm S D of three replicates

3.2. Antibacterial potential

The antibacterial potential of each variety and each polarity extract of unripe banana was determined by the diffusion method reported (Rao et al., 2016; Reinisalo et al., 2015). The potential of various polarities extracts was determined through the gel diffusion method against Gram (+ and -) antibacterial strains. Each extract of samples from both regions at four different concentrations was used to evaluate the antibacterial potential. All extracts of unripe bananas displayed different ranges of zones of inhibition and the results were presented in Adinarayana. Among the gram (+ and -) antibacterial strains, all gram (-) antibacterial strains gave more zone of inhibition compare to gram (+) antibacterial strains. On the other hand, non-polar extract gave the highest inhibition compared then other extracts (Table 2).

3.3. Cytotoxic potential

All six extracts with different polarities showed substantial cytotoxic potential against the artemia cysts reported by several authors (Rehab & Hossain, 2016). The percentage of mortality (%) and IC_{50} values are shown in Table 3. Among the extracts with different polarities, the non-polar hexane extract gave more IC_{50} compared to other extracts. On the other hand, non-polar extract gave the highest inhibition compared then other extracts (Table 3).

3.4. Discussion

Since old times, the world population has been using different herbal therapies as a safe and primary health care system to treat diverse diseases. Researchers are working on the available natural resources to search for pharmacologically active compounds, specially antibiotics and anticancer drugs. The selected banana crop is one of the most valuable nutritional agricultural crops. It gives us instant energy and has several medicinal benefits. Banana is mainly used to treat indigestion, hypertension, constipation, GI problems and diarrhea (Liu, 2004; Mallikarjuna & Jyothirmay, 2011; Natcharee & Sudip 2011; Latifa & Hossain, 2019; Serafino et al., 2008; Weli et al., 2014). Omani communities also use the fruit to treat these problems. In this context, this present study, we intend to assess the antibacterial and cytotoxic potential of various species of local unripe banana (Sohar and Dhofar).

The antibacterial potential of six extracts varied polarities at four numerous concentrations from samples from both areas was determined against four Gram (+ and -) antibacterial strains. All culture antibacterial strains were available in our laboratory. The experimental results from the six different extracts showed that all six extracts give reasonable antibacterial potential against the applied antibacterial strains at varied concentrations in the range of 0-11.5 mm.

Table 2. Antimicrobial activity of different crude extracts from Sohar and Dhofar unripe banana samples

Bacteria	Extract	Hexane		Chloroform		Ethyl acetate		Butanol		Methanol		Water	
		Conc. (mg/ml)	Sohar (mm)	Dhofar (mm)	Sohar (mm)	Dhofar (mm)	Sohar (mm)	Dhofar (mm)	Sohar (mm)	Dhofar (mm)	Sohar (mm)	Dhofar (mm)	Sohar (mm)
<i>E. coli</i> (Code no. 337)	2	11±0.12	10.5±0.56	13±0.14	9±0.42	9±0.12	7.5±0.55	7±0.15	9±0.20	7±0.12	8±0.17	7±0.13	7.5±0.07
	1	11±0.43	10±0.23	8±0.11	8.5±0.33	8±0.10	7±0.18	7±0.43	8.5±0.07	8±0.23	7.5±0.10	6±0.90	7±0.21
	0.5	9.5±0.89	7±0.78	8±0.09	6.5±0.41	7.75±0.11	6.5±0.23	7±0.55	8±0.65	7.15±0.15	6.5±0.15	7±0.10	6.5±0.57
	0.25	9±0.15	6.5±0.23	7±0.16	6±0.10	9±0.07	6±0.32	7±0.08	7±0.14	7±0.10	nd	6±0.19	6±0.16
Control	3	12±0.32	8±0.47	30±0.10	25±0.16	11±0.23	29±0.21	10±0.25	9±0.18	7±0.10	nd	9±0.12	22±0.13
<i>H. influenza</i> (Code no. 236)	2	7±0.76	8±0.28	8±0.19	7.5±0.19	8±0.15	7.5±0.10	8±0.18	7.5±0.15	8±0.32	8.5±0.09	9±0.15	nd
	1	6±0.19	7.5±0.26	6±0.22	7±0.31	8±0.78	7±0.19	8±0.42	7.15±0.11	7±0.42	8.15±0.54	9±0.08	nd
	0.5	nd	7±0.55	8±0.41	6.5±0.64	7.5±0.52	6.5±0.11	7±0.08	7.15±0.17	7±0.12	8±0.22	6±0.10	nd
	0.25	nd	7±0.16	8±0.23	6.5±0.55	7±0.10	6.5±0.45	6±0.09	7±0.19	nd	nd	6±0.54	nd
Control	3	33±0.18	26±0.21	28±0.32	27±0.14	27±0.10	27±0.56	28±0.23	27±0.09	29±0.15	33±0.32	30±0.87	25±0.23
<i>S. aureus</i> (Code no. 207)	2	6±0.90	8±0.12	nd	9±0.17	6±0.55	10±0.23	8±0.27	nd	8±0.10	10±0.10	8±0.10	nd
	1	0±0.39	7±0.82	nd	8.5±0.29	nd	9±0.91	6±0.14	nd	6±0.35	8±0.15	7±0.10	nd
	0.5	0±0.55	7±0.29	nd	6.5±0.18	nd	9±0.12	6±0.17	nd	6±0.34	7.5±0.19	nd	nd
	0.25	0±0.72	6±0.65	nd	6±0.15	nd	9±0.16	6±0.19	nd	6±0.18	nd	nd	nd
Control	3	24±0.15	31±0.34	26±0.17	29±0.10	29±0.23	29±0.10	27±0.10	25±0.89	27±0.13	nd	29±0.16	29±0.13
<i>S. pneumoniae</i> (Code no. 257)	2	8±0.82	7.5±0.41	7±0.34	7.5±0.06	7±0.11	8±0.22	nd	7±0.10	8±0.21	7±0.11	nd	7±0.09
	1	7±0.24	7±0.23	7±0.14	7±0.18	6±0.18	6.15±0.10	nd	6.75±0.22	7±0.19	7±0.10	nd	6.5±0.10
	0.5	6±0.12	6.5±0.24	6±0.10	6±0.52	6±0.25	6±0.09	nd	6.5±0.37	7±0.10	nd	nd	nd
	0.25	0±0.12	6.5±0.27	nd	6±0.21	6±0.72	0±0.65	nd	6±0.13	nd	nd	nd	nd
Control	3	31±0.12	27±0.56	27±0.18	34±0.32	33±0.12	40±0.15	31±0.22	34±0.17	26±0.12	30±0.17	30±0.12	26±0.19

nd= not detected; Each value is a mean of three biological replicates

Table 3. Percentage of mortality and lethal concentration (IC₅₀) of different polarities Sohar and Dhofar unripe banana samples

Crude extract	Conc µg/ml	Mortality (%)		LC ₅₀ (µg/ml)	
		Sohar	Dhofar	Sohar	Dhofar
Hexane	500	100	100		
	100	80	80	22.39±011	27.88±017
	50	60	50		
	10	30	10		
	Control	0	0		
Chloroform	500	100	100		
	100	70	80	29.74±0.14	29.16±0.10
	50	50	40		
	10	20	20		
	Control	0	0		
Ethyl acetate	500	100	100		
	100	60	50	36.68±0.22	49.32±0.16
	50	50	40		
	10	10	10		
	Control	0	0		
Butanol	500	100	100		
	100	90	80	20.12±0.10	26.19±0.57
	50	60	50		
	10	30	20		
	Control	0	0		
Methanol	500	100	100		
	100	80	70	29.15±0.18	27.86±0.23
	50	40	50		
	10	20	30		
	Control	0	0		
Water	500	100	100		
	100	80	50		
	50	50	40	27.94±0.25	24.22±0.18
	10	10	20		
	Control	0	0		

Each value is a mean ±SD of three biological replicates

Among the six unripe banana extracts, only the chloroform extract showed the maximum potential in the samples collected from Sohar against *E. coli* at 2 mg/ml. The other five extracts from both regions displayed average potential against *E. coli* at several varied concentrations. Conversely, the water extract from Dhofar did not show potential against both *H. influenza* and *S. aureus* at any prepared concentrations. In addition, the chloroform

extract from Sohar banana also did not show any potential against *S. aureus* at any prepared concentrations. It means that the antibacterial activity of the plant extracts largely depends on numerous factors such as chemical ingredients, doses of extract, sensitivity of the antibacterial strains and the types of antibacterial strains used in the experiment. Our experiment results indicated that all six extracts varied polarity gave dissimilar potential that means not all

polarities extracts from both types of unripe bananas contain enough number of chemical ingredients that can actively participate for antibacterial potential. Several other related reports available on antibacterial potential of various polarities extracts showed that the unripe banana extracts gave reasonable activity against almost all gram (+ and -) antibacterial strains (Latifa & Hossain, 2019). The obtained results also indicated that not all six polarities extracts are significant against the applied gram (+ and -) antibacterial strains. The reason for variation of antibacterial results could be the chemical ingredients in the extracts or the sensitivity of the applied microbes. On the other hand, the cytotoxic potential of all six polarities banana extracts did not kill all tested nauplii (mortality 100%) at the concentration of 500 ug/ml. The mortality (%) of our experiment for all six polarities extracts at all prepared concentrations was given in Table 3. The maximum cytotoxic potential was obtained in butanol extract from Sohar and the water extract of Dhofar, but less potential was obtained in ethyl acetate extract. In this present experiment, it indicates that there was a significant correlation between the concentration and mortalities. The mortality (%) is increased with the increasing concentrations of the banana extract. Our results are completely different from what has been reported on various polarity extracts of unripe banana samples collected from elsewhere (Serafino et al., 2008; Weli et al., 2014; WHO, 1993; Yusoff & Adlin, 2008; Zafar et al., 2011; Al Alawi et al., 2018). The variation of mortality (%) and LC₅₀ value could have been caused by the differences in the procedures for evaluating cytotoxicity. In our present study, BST assay was used however, other investigators used *in-vitro* or *in-vivo* based assay.

4. Conclusions

The aim of this current study was to evaluate the antibacterial and cytotoxic potential of unripe bananas from Sohar and Dhofar by gel diffusion and BSL bioassays. Six different polarities extracts showed substantial

antibacterial potential against the Gram (+ and -) antibacterial strains. In addition, all six polarities extracts also indicated moderate cytotoxic potential against BSL assay. The maximum potential extracts can be used as a natural safe medicine to treat different human ailments. Further extensive studies will be planned for the isolation, characterization and evaluation of the pharmacologically active pure ingredients and their diverse action.

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