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PHYTOCHEMICAL SCREENING AND BIOASSAY OF THE ANTIBACTERIAL ACTIVITY OF *Hylocereus undatus* and *Hylocereus polyrhizus* FRUIT PEEL

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ABSTRACT

This study was conducted to screen the presence of tannins, steroids, saponins, flavonoids, alkaloids, terpenoids, and cardiac glycosides in ethanol and hot water extracts of *Hylocerus undatus* and *Hylocerus polyrhizus* fruit peels. Antibacterial properties of the aforementioned dragon fruit peels as protectant and eradicant against *Escherichia coli* 25922 and *Staphylococcus aureus* 25923 were also evaluated.

Phytochemical screening showed that *H. undatus* and *H. polyrhizus* fruit peel extracts contains terpenoids and cardiac glycosides. While steroids and saponins are present only in *H. undatus* and *H. polyrhizus* ethanol extracts.

Antibacterial activity as eradicant of ethanol extract of *H. undatus* and *H. polyrhizus* fruit peel and protectant activity of *H. undatus* ethanol extract, *H. polyrhizus* fruit peel hot water and ethanol extracts against *E.coli* at 12, 24 and 36 hrs of incubation were observed.

Keywords: antibacterial, dragon fruit, eradicant, phytochemical, protectant

INTRODUCTION

Dragon fruit or Pitaya was first introduced in the Philippines in 1990s in Ilocos region where it was locally called as Sanaita [1]. Dragon fruit is a perennial, epiphytic, climbing cactus with a triangular fleshy jointed stems which belongs to family Cactacea and of genus *Hylocerous* [2, 3]. There are three varieties of dragon fruit which include *Selenicereus megalathus* or white flesh with yellow peel dragon fruit, *Hylocereus undatus* or white-flesh with red peel dragon fruit and *Hylocereus polyrhizus* or red-flesh with red peel dragon fruit. They are rich in vitamins, fiber, phosphorus, calcium, magnesium, calcium, magnesium, phytochemicals and antioxidants [4]. Accordingly, Yong [5] revealed that dragon fruit is also known to possess medicinal properties that could prevent cancer, diabetes and neutralizes toxins in the body. It is also helpful in reducing blood sugar levels in people suffering from type 2 diabetics [6, 7, 8].

In the past decade interest on the topic of antibacterial property of plant extracts which can be attributed to their bioactive compounds has been growing and researchers attributed this to the presence of several bioactive compounds [9]. Phytochemicals such as carotenoids, polyphenols and anthocyanin that are abundantly present in fruits and vegetables such as tomatoes, grapes, pomegranates and strawberries are gaining lot of interest due to their functional property [10, 11, 12]. Furthermore, studies of Ahmad and Beg [13] Cushnie and Lamb [14] showed anti-microbial potentials of polyphenols such as flavonoids and tannins have shown very promising results in combating bacteria, fungus and viral.

Several researches have already proved the nutritional as well as the medicinal importance of the dragon fruit pulp, however, dragon fruit peel has been under used and its importance is yet to be explored. Hence, the study was conducted to screen the phytochemical constituents of the dragon fruit peel and to determine the antibacterial potentials as protectant and eradicant against *E. coli* and *S. aureus*.

MATERIALS AND METHODS

Collection and preparation of the plant materials

Fruit peel of matured and ripened *H. undatus* and *H. polyrhizus* were collected, shredded, dried and were crushed into powdered form.

Preparation of peel extracts

Fruit peels of *H. undatus* and *H. polyrhizus* were extracted using hot water and ethanol as solvents in a ratio of 1:4.

Preparation of the test organism

Using sterile loop, bacteria were inoculated to the sterilized test tube with nutrient broth and were stored at room temperature for 24 hours. For the determination of the bacterial cell density ($1.5x10^8$ cells /ml), McFarland 0.5 was used.

Preparation of the assay plates and paper disc

Mueller Hinton Agar II was prepared by mixing 19 g of Mueller Hinton Agar II with 500 ml of distilled water in an Erlenmeyer flask and was dissolved using hot plate. Then, it was sterilized at 121°C, 15 psi for 15 minutes. After the sterilization, the agar was distributed in the assay plates with approximately 20 ml each.

For the preparation of paper disc, Whatman Filter paper no. 1 was punched with paper puncher. Then, the paper discs were placed in Petri plates and were sterilized at 121°C, 15 psi for 15 minutes.

Bio assay testing

Protectant and eradicant test was carried out in the study. For the eradicant test, the sterile paper discs were soaked separately in a 1 ml of hot water and ethanol extracts of dragon fruit peel for 30 minutes, and was dried inside the chamber for 1 hour. For the bacterial inoculum, 0.2 ml of the inoculum was spread evenly in the assay plates using L-rod. Then the discs treated with different extracts were seeded equidistantly using a sterile forceps. The plates were properly labelled and incubated at room temperature in an inverted position. The zone of inhibition was measured at 12, 24, and 36 hrs of incubation using digital vernier caliper.

Meanwhile, for the protectant test, the paper disc was soaked in the bacterial suspension. The plates were then poured with 0.2 ml of fruit peel extracts. The paper discs were seeded equidistantly in the plates together with the positive and negative control and were stored at room temperature. Zone of colonization was measured after 12, 24, and 36 hours of incubation.

Screening of phytochemical composition of dragon fruit peel

Screening of phytochemical constituents were carried out following the standard methods described in Laboratory Manual for the UNESCO (1990). The various phytochemical constituents tested were the following: tannins, steroids, saponins, flavonoids, terpenoids, cardiac glycosides, and alkaloids.

Test for tannins

An aliquot of 0.5 mL extract of the selected toxic plants were added to 10 mL of distilled water on a test tube and was filtered using a filter paper. Two mL of 5% Ferric Chloride (FeCl) was added to the filtered sample. Brownish green or blue black coloration was observed for the presence of tannins.

Test for steroids

Two milliliters of acetic anhydride was added to a 5 mL extract of plant sample with 2 mL of Sulfuric Acid (H_2SO_4). Violet to blue or green precipitate was observed for the presence of steroids.

Test for saponins

A volume of 0.5 mL of extract was added to 10mL distilled water and was shaken vigorously to obtain a stable persistent froth. Persistent frothing indicated the presence of saponin.

Test for flavonoids

Few drops of 1% Ammonia (NH_3) solution was added to 5 mL extract of plant sample in a test tube. Yellow coloration indicated the presence of flavonoids.

Test for terpenoids

Five milliliters extract of plant sample was added with 2 mL Chloroform ($CHCl_3$) in a test tube. Then, 3 mL of Sulfuric Acid (H_2SO_4) was added carefully to the mixture to form a layer. The formation of the reddish brown interface indicates for the presence of terpenoids.

Test for cardiac glycosides

One milliliter of concentrated Sulfuric Acid (H_2SO_4) was prepared in a test tube. Five milliliters of extract from the plant sample was mixed with 2 mL of glacial acetic acid (HCH_3CO_2) containing one drop of Ferric Chloride ($FeCl_3$). The mixture was added carefully to 1 mL of concentrated H_2SO_4 so that the concentrated H_2SO_4 was underneath the mixture. The appearance of brown ring indicated the presence of cardiac glycosides.

Test for alkaloids

Five milliliters of the extract was prepared in a beaker and 200 mL of 10% HCH_3CO_2 in ethanol (C_2H_5OH) was added. The mixture was filtered and the extract was allowed to become concentrated in a water bath until it reached one fourth of the original volume. Concentrated NH_4OH was added. Formation of the white precipitate or turbidity indicated the presence of alkaloids (Trease and Evans, 1983).

RESULTS

Phytochemical Composition of the Two Species of Dragon Fruit Peel Extracts

Phytochemicals are nonnutritive plant chemicals that have protective or disease

preventive properties against pests, pathogens, fungi and animals [16, 17, 18]. Plant produces these chemicals to protect itself, but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each works differently [19]. There is a wide diversity of compounds, especially secondary metabolites, found and isolated from plants and studies have shown that these compounds have anticancer, antibacterial, analgesic, anti-inflammatory, antitumor, antiviral and many other activities to a greater or lesser extent [20]. Distinguished examples of these phytochemical compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides, stilbenes, tannins, nitrogen compounds (alkaloids, amines, betalains), terpenoids and some other endogenous metabolites [21].

The effect of water and ethanol as solvents in the extraction of dragon fruit peel was clearly demonstrated in the study. According to Sineiro et al. [22], solvent and process variables are important to optimize extraction yield, similarly polarity of the solvent determines the qualitative and quantitave property of the extracts. Results of phytochemical analyses are presented in the Table 1. The absence of tannins, flavonoids and alkaloids in all the tested plant extracts was revealed. Meanwhile, terpenoids and cardiac glycosides were present in both ethanol and hot water extracts of *H. undatus* and *H. polyrhizus* fruit peel. While steroids and saponins were also present in *H. undatus* and *H. polyrhizus* ethanol extracts.

Results of the study coincides with the study of Mohammedi and Atik [23], wherein more phytochemicals were found in plants extracted with alcohol as solvent. According to Perva-Uzunalic [24], and Mohamedi and Atik [23] phenolic extracts of plants are always a mixture of different classes of phenols, which are selectively soluble in the solvents and alcoholic solution provides satisfactory results for the extraction process and only water soluble compounds are extracted with water.

Bio assay of the antibacterial potentials of *Hylocereus undatus* and *Hylocereus polyrhizus* fruit peel extracts

Eradicant activity of *H. undatus* and *H. polyrhizus* fruit peels against *E. coli*

Among the four fruit peel extracts tested only the ethanol extracts of *H. undatus* and *H. polyrhizus* fruit peels produced zones of inhibition after 12, 24 and 36 hrs of incubation (Table 2). At all incubation

period, streptomycin treated discs had the highest mean zone of inhibition of 28.14 mm, 32.41 mm and 35.53 mm respectively. This was followed by *H. polyrhizus* fruit peel ethanol extracts with 25.30 mm, 28.24 and 28.21 at 12, 24 and 36 hrs of incubation while *H. undatus* fruit peel ethanol extracts registered mean zones of inhibition of 20.81 mm, 25.09 mm and 25.09 mm respectively. Statistical analysis revealed no significant differences among the means of the zones of inhibition produced by streptomycin sulfate as to the zones of inhibition observed in ethanol extracts of *H. undatus* (at 12 and 24 hrs) and *H. polyrhizus* (at 12 and 24 hrs). This indicates the antibacterial activity as eradicant of the aforementioned extracts which is comparable to the effect of the antibacterial drug. Interpretatively, streptomycin sulfate, *H. undatus* and H. *polyrhizus* ethanol extracts exhibited a very active response against *E. coli* at all incubation period. Meanwhile, no zones of inhibition were recorded in *H. undatus* and *H. polyrhizus* hot water extracts which were also regarded as an inactive response to *E. coli*.

Protectant activity of *H. undatus* and *H. polyrhizus* fruit peels against *E. coli*

For the protectant test, zones of colonization were measured. Zones of colonization indicate the inability of the dragon fruit peel extracts to control the growth of the test pathogen. Whereas, the lesser or the absence of zone of colonization by the test pathogens in plates treated with the dragon fruit peel extracts, the greater the antibacterial activity as protectant of the dragon fruit peel extracts against *E. coli* and *S. aureus*.

Results of the protectant test against *E.coli* is presented in Table 4. No bacterial growth was observed in plates treated with *H. undatus* fruit peel ethanol extract, *H. polyrhizus* fruit peel hot water and ethanol extracts. This greatly indicates the antibacterial activity of *H. undatus* ethanol extract, *H. polyrhizus* hot water and ethanol extracts against *E. coli*, thus preventing bacterial colonization. Meanwhile, *H. undatus* fruit peel hot water extracts failed to prevent the growth of *E. coli* with mean zones of colonization of 12.97 mm, 15.17 mm and 18. 62 mm at 12, 24 and 36 hrs of incubation.

Protectant Test of Different Dragon Fruit Peel Extracts against *S. aureus*

Cited in Table 5 are the mean zones of colonization of *S. aureus* at 12, 24 and 36 hrs of incubation. Zones of colonization were observed in all plates treated with fruit peel extracts. While at 12 and 24 hrs of incubation, *H. undatus* ethanol extracts;

ethanol and hot water extracts of *H. polyrhizus* were comparable to the effect of Streptomycin sulfate against *S. aureus* which suggests their protectant potentials against *S. aureus*.

Table 1: Phytochemical composition of the ethanol and hot water extracts of *H. polyrhizus* and *H. undatus*

Phytochemical composition	*H. undatus* hot water extract	*H. undatus* ethanol extract	*H. polyrhizus* hot water extract	*H. polyrhizus* ethanol extract
Tannins	-	-	-	-
Steroids	-	+	-	+
Flavanoids	-	-	-	-
Saponins	-	+	-	+
Terpenoids	+	+	+	+
Alkaloids	-	-	-	-
Cardiac glycosides	+	+	+	+

(+) = presence of phytochemical; (-) = absence of phytochemical

Table 2: Diameter of zones of inhibition (mm) of the different treatments against *E. coli* after 12, 24, and 36 hours of incubation

TREATMENT	ZONE OF INHIBITION (mm) 12 Hrs	24 Hours	36 Hours	INTERPRETATION
Streptomycin sulfate	28.14 ^{b}	32.41^{c}	35.53^{c}	Very active
Distilled water	6.00^{a}	6.00^{a}	6.00^{a}	Inactive
H.undatus hot water extracts	6.00^{a}	6.00^{a}	6.00^{a}	Inactive
H.undatus ethanol extracts	20.81^{b}	25.09^{b}	25.09^{b}	Very active
H. polyrhizus hot water extract	6.00^{a}	6.00^{a}	6.00^{a}	Inactive
H. polyrhizus ethanol extract	25.30^{b}	28.24^{bc}	28.21^{b}	Very active

***Means with the same letter superscript are not significantly different at 5% level of signfcance by Duncan Multiple Range Test (DMRT)**

Table 3: Diameter of zones of inhibition (mm) of the different treatments against *S.aureus* after 12, 24, and 36 hours of incubation

TREATMENTS	ZONE OF INHIBITION (mm) 12 hrs	24 hrs	36 hrs	INTERPRETATION
Streptomycin sulfate	22.26^{b}	22.21^{b}	22.81^{b}	Very active
Distilled water	6.00^{a}	6.00^{a}	6.00^{a}	Inactive
H.undatus hot water extracts	6.00^{a}	6.00^{a}	6.00^{a}	Inactive
H.undatus ethanol extracts	6.35^{a}	6.14^{a}	6.00^{a}	Inactive
H. polyrhizus hot water extract	6.00^{a}	6.00^{a}	6.00^{a}	Inactive
H. polyrhizus ethanol extract	7.89^{a}	7.26^{a}	6.23^{a}	Inactive

***Means with the same letter superscript are not significantly different at 5% level of significance by Duncan Multiple Range Test (DMRT)**

Table 4: Diameter of zones of colonization (mm) of *E.coli* in different treatments after 12, 24, and 36 hours of incubation

TREATMENTS	ZONE OF COLONIZATION (mm)		
	12 Hours	24 Hours	36 Hours
Streptomycin sulfate	6.00[a]	6.00[a]	6.00[a]
Distilled water	7.65[a]	11.48[b]	15.46[b]
H.undatus hot water extracts	12.97[b]	15.17[c]	18.62[c]
H.undatus ethanol extracts	6.00[a]	6.00[a]	6.00[a]
H. polyrhizus hot water extract	6.00[a]	6.00[a]	6.00[a]
H. polyrhizus ethanol extract	6.00[a]	6.00[a]	6.00[a]

***Means with the same letter superscript are not significantly different at 5% level of significance by Duncan Multiple Range Test (DMRT)**

Table 5: Diameter of zones of colonization (mm) *S.aureus* in different treatments after 12, 24, and 36 hours of incubation

Treatment	ZONE OF COLONIZATION (mm)		
	12 Hours	24 Hours	36 Hours
Streptomycin sulfate	6.00[a]	6.00[a]	6.00[a]
Distilled water	9.62[a]	15.59[a]	20.08[bc]
H.undatus hot water extracts	6.00[a]	8.28[a]	9.20[ab]
H.undatus ethanol extracts	34.05[b]	38.26[b]	40.42[d]
H. polyrhizus hot water extract	9.36[a]	15.29[a]	19.29[bc]
H. polyrhizus ethanol extract	10.71[a]	13.69[a]	22.06[c]

***Means with the same letter superscript are not significantly different at 5% level of significance by Duncan Multiple Range Test (DMRT)**

DISCUSSIONS

These findings coincides with the study of Nurmahani et al. [25], wherein *H. polyrhizus* and *H. undatus* fruit peel ethanol extracts inhibited the growth of nine species of bacterial pathogens including *E. coli*. Also, in a study of Phongtongpasuk et al. [26], the antibacterial activity of silver nanoparticles from dragon fruit peel extracts against selected human pathogenic bacteria (*E. coli*, *P. aeruginosa*, and *S. aureus*) was elucidated by disc diffusion assay. Additionally, its antibacterial activity against *E. coli* can be attributed to the presence of phytochemicals such as cardiac glycosides, saponins, terpenoids and steroids of the *H. undatus* and *H. polyrhizus* fruit peel ethanol extracts. These secondary metabolites in fruits and vegetables have shown very promising results in combating bacteria, fungus and viral [13, 14, 27, 28, 29]. Accordingly, these metabolites cause damage to pathogens by inhibiting microbial protein and nucleic acid synthesis, disrupting microbial membrane structure and function, or blocking metabolic pathways through inhibition of key enzymes [30]. They also serve a defense mechanism against invasion by many microorganisms, insects and other herbivores [31]. Compounds such as cardenolides, flavonoids, resins, saponins and tannins have been shown to have healing properties against most disease causing organisms. These properties include

antioxidant activity, anti-allergic, anti-inflammatory and many others [32]. Saponins from a variety of plant sources have been shown to have pharmacological properties and are the major components of many plant-derived drugs and folk remedies [33]. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell. Plant terpenoids can be used enormous for their aromatic qualities. Terpenoids can play an important role in traditional medicines and are under investigation for antineoplastic, antibacterial and other pharmaceutical functions [34, 35].

CONCLUSION

Results of the study revealed the presence of terpenoids, cardiac glycosides, steroids and saponins in *H. undatus* and *H. polyrhizus* fruit peel ethanol extracts. Antibacterial activity of *H. undatus* and *H. polyrhizus* fruit peel ethanol extract as eradicant and protectant against *E.coli,* while inactive response were noted against *S. aureus*. Thus further studies must be carried out for the potential utilization of dragon fruit peel as antimicrobial agents.

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