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Characterization and Antimicrobial activity of different varieties of *Citrullus lanatus* rind of Balochistan

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Abstract

Phytotherapy had been overlooked due to synthetic medications, but recently they have regained prominence. Plants contain extractable organic substances in quantities adequate to be economically useful as raw materials for various technological, scientific, and pharmaceutical applications. Agricultural wastes, such as the rind of a watermelon, would serve as exceptional source of natural products. This study shows that three different varieties of rind of watermelon produced in Balochistan are rich in some bioactive compounds. The air-dried watermelon rinds were extracted with n-hexane, methanol and ethanol, using maceration and Soxhlet extraction procedures. The phytochemical findings of methanolic and ethanolic extracts displayed the presence of carbohydrates, amino acids, proteins, alkaloids, steroids, phytosterols, flavonoids, saponins, and glycosides. The n-hexane extracts were subjected to the

antibacterial assay via disk diffusion test on gram positive and gram negative bacteria that is Staphylococcus Aureus and Escherichia coli. The study confirmed that Citrullus lanatus rind exhibits antibacterial activity against Staphylococcus Aureus and E.coli. The Summer flavor 840 watermelon rind showed the highest antibacterial activity with zone of inhibition (ZOI) 5mm against E.coli and 10mm against Staphylococcus Aureus whereas Charleston Gray variety produced ZOI 5mm against E.coli however no activity has been displayed against Staphylococcus Aureus while on the contrary Crimson Sweet watermelon rind induced no activity against E.coli whereby ZOI of 5mm was formed against Staphylococcus Aureus.

Keywords: Phytotherapy, Agricultural waste, Citrullus lanatus, Rind, Phytochemicals, Qualitative screening, Antibacterial activity.

INTRODUCTION

As the world's population grows, so does the demand for production of food, resulting in an increase of waste generation. Consumption and processing of vegetables and fruits produce 25–30% solid waste from their inedible parts. Huge production and mismanaged handling of waste have led to massive deposition, posing severe environmental hazards, including soil contamination, smoke and the release of greenhouse gases, when these residues are decomposed, dumped or burnt (Kearney 2010).

To prevent harmful effects of solid waste on the environment, these agricultural waste products should be utilized for their pharmacological activities. The concentrations of phytochemicals in agro-waste are considerably higher than their respective edible tissues, indicating these wastes to be a major source of bio-active compounds of supreme importance in pharmaceutical industry (Firn 2010).

Presence of Phytochemicals in plants imparts protective and disease preventive characteristics. Apart from protection, they provide the plant with aroma, colour, and flavour. Fruits and herbs have many phytochemicals, each works differently (Kumar et al. 2009). These biologically significant components exhibit anti-inflammatory,

antioxidative, anti-cancer, immune system stimulation and antimicrobial effects. Moreover, they also reduce the prevalence of cardiac diseases, oxidative stress and diabetes (Wadhwa et al. 2015).

The drug resistance is dangerously rising worldwide leading to high mortality and prolong illness. (Boucher et al. 2009). Increasing antimicrobial resistance is threatening our abilities, making it difficult and sometimes impossible to cure common infectious diseases. The current situation demands the evolution of new drugs. The presence of different secondary metabolites in plant originated drugs make them an alternative for the treating the microbial infections (Iwu et al. 1999).

Watermelon (*Citrullus lanatus*) is the prominent member of the Cucurbitaceae family (Dane and Lu 2007). This family ranks among the highest of plant families, as most of its species serve as plant-based foods. Cucurbits are grown in temperate regions. The other plants of this family include pumpkins, some gourds, squash, cucumber, and melons. Watermelon is a thirst-quenching popular summer fruit originated from Africa. It contains 92% water along with plenty of minerals, vitamins, and other compounds. About 50 different varieties of watermelon have been cultivated, varying in the bioactive compounds.

An average watermelon yields about 55.3% fruit, 10.4% seeds and 31.5% rind. Although seeds and rind contain a lot of nutrients, still they are considered as waste and are usually discarded generating a huge amount of waste (Oberoi and Sogi 2017). Previously there have been a lot of studies on watermelon fruits, mostly on the juice, pulp or seed, however exploration of rind and peel is still insufficient. Therefore, the present studies focuses on the agro-waste, the watermelon rind, to identify various secondary metabolites present in them, and its potential to serve as a novel antibiotic. The aim of this study is to convert a waste to wonder.

MATERIAL AND METHODS

Collection of Plant Material

Three different varieties of watermelon were purchased in July from the local vendors of Quetta, Balochistan. The selected varieties were identified as Charleston Gray, Crimson Sweet and Summer Flavor

840 in the Department of Botany, University of Balochistan. The flesh and peel were removed, and fresh white rind was collected. The rinds were chopped into small pieces and were kept in the covered environment for 10 days.

Charleston Gray Watermelon (Elongated, light green)

Summer Flavor 840 Watermelon (Elongated, dark green strips)

Crimson Sweet Watermelon (Round, dark green strips)

Figure 1: Different varieties of Watermelon cultivated in Balochistan

Sample extraction

The dried rinds were converted to a fine powder with automatic grinder and accurately weighed using a digital balance. All three samples were separately stored in plastic seal bags, before further analysis. Extraction was done by two methods, through Soxhlet extraction and maceration. In Soxhlet extraction 30 g of each rind sample was extracted with n-hexane. The extraction took 6 hours each time. Extraction through maceration was done with alcoholic solvents methanol and ethanol, 50 g dry sample of each variety was macerated in 100 ml solvent for three days at room temperature and was shaken daily. The procedure was repeated thrice. The extracts were filtered and the filtrate was concentrated using rotary evaporator at 60℃ to obtain the crude extract. These extracts were stored in dark bottles.

Qualitative screening of Phytochemicals

a) Detection of Carbohydrates

The presence of carbohydrates in pure methanol and ethanol extracts of the three varieties of *Citrullus lanatus* rind were determined by Molisch's test, Fehling's test and Benedict's test (De Silva et al. 2017).

b) Detection of Protein and Amino acids

Ninhydrin test for amino acid (Yadav and Agarwala 2011), and Biuret and Xanthoproteic tests for protein detection were performed as stated by (De Silva et al. 2017).

c) Detection of Alkaloids

The standard procedures of Mayer's test, Wagner's test, Dragendorff's test and Hager's tests were followed to confirm the presence of alkaloids (De Silva et al. 2017).

d) Detection of Flavonoids

To analyse the existence of flavonoids alkaline reagent test and lead acetate test were performed (De Silva et al. 2017).

e) Detection of Saponins

The presence of saponins in the extracts were determined as stated by (Delta 2011).

f) Detection of Tannins

Ferric chloride test was performed to monitor presence of Tannins (Shaikh and Patil 2020).

g) Detection of Steroids

The determination of steroids in the extracts was carried out as per standard procedures (Roghini and Vijayalakshmi 2018).

h) Detection of Glycosides

Salkowski's test, Keller-Killani test and Liebermann's test were performed to evaluate the presence of glycosides (Gul et al. 2017).

i) Detection of Phytosterol

To evaluate the presence of phytosterols in the extracts Liebermann-Burchard test and Salkowski's test were performed (De Silva et al. 2017).

Antibacterial Assay

Agar disk diffusion method

The extracts of watermelon rind obtained from the Soxhlet extractions were tested for their antibacterial activity against the clinical isolates: Escherichia coli (E.coli) and Staphylococcus Aureus. Antibacterial test was carried out by applying the Kirby-Bauer disk diffusion method with few modifications. 38 g of Mueller Hinton Agar (MHA) was dissolved in 1000 ml distilled water to prepare bacterial culture plates. The mixture was autoclaved at 121°C for 15 minutes. Media was allowed to cool and was poured in the petri plates. With a sterile

swab sub-cultured bacterial strains were transferred into petri plates and were allowed to sit at room temperature. 6mm round filter paper discs were loaded with 100 µL watermelon rind extracts. These discs were placed on the agar plate along with positive and negative controls. For both the tests, Ciprofloxacin was used as positive control whereas NaCl was used as negative control. Each plate was appropriately labelled for the organism to be tested. Plates were incubated for 24 hours at 37℃. Antibacterial activity was measured in diameter of the zone produced around the discs.

RESULT AND DISCUSSION

Phytochemical screening

The qualitative screening of pure methanolic and ethanolic extracts of *Citrullus lanatus* rind is displayed in Table 1. The study showed that several phytochemical that includes carbohydrates, proteins, alkaloids, flavonoids, steroids, saponins, phytosterols and glycosides were present in all the varieties. However, tannins were not present in any sample.

Carbohydrates

Carbohydrates in the methanolic and ethanolic extracts were determined by applying Molisch's, Fehling's, and Benedict's test. With the formation of a violet ring on adding sulphuric acid in the Molisch's test, the presence of carbohydrates was confirmed. Whereas in Fehling's and Benedict's test the formation of brick red and red precipitates confirmed presence of carbohydrates. These results are in accordance with the result based on the findings of Al-Sayed and Ahmed 2013.

Proteins and Amino acids

The presence of proteins and amino acids were analysed by applying Xanthoproteic, Biuret, and Ninhydrin tests. The formation of a violet-coloured product in Biuret test and yellow product in Xanthoproteic test confirmed the presence of protein while in Ninhydrin test appearance of purple colour indicated the presence of amino acids (Table 1). These results are consistent with the previous findings based on the study of Al-Sayed and Ahmed 2013.

Alkaloids

Mayer's, Hager's, Wagner's and Dragendorff's tests were performed to confirm the presence of Alkaloids. In Mayer's test creamy precipitates appeared, while reddish-brown precipitates in Wagner's and Dragendorff's tests indicate the presence of alkaloids. In Hager's test yellow precipitates confirmed the presence of alkaloids (Table 1). The results are consistent with the results of Harith et al. 2018.

Flavonoids

Flavonoids content was monitored by alkaline-reagent test and lead acetate test. The intense yellow colour turned colourless in alkaline-reagent test detecting the presence of flavonoids in the samples. Whereas lead acetate test showed yellow precipitation confirmed the presence of flavonoids (Table 1). These results are in accordance with the results of Osinubi et al. 2020.

Saponins

The formation of a stable foam revealed that saponins are present in the methanolic and ethanolic extracts (Table 1), which is consistent with previous study of (Oluyori et al. 2017).

Tannins

The results of ferric chloride test for tannins detection evaluated that tannins are absent as there was no change colour in methanolic and ethanolic extract, results are consistent with the previous study of Harith et al. 2018.

Steroids

Steroid identification test of methanolic and ethanolic extracts showed the appearance of red or green-yellow color ring confirming the presence of steroids, which is consistent with previous study of Osinubi et al. 2020.

Glycosides

The presence of glycosides was determined by performing Keller-Killani, Salkowski's and Liebermann's test. Appearance of brown ring on addition of sulphuric acid in Keller-Killani test, reddish brown ring in Salkowski's test and green colour of extracts in Liebermann's test indicates the presence of glycoside. The results were in accordance with the results of Harith et al. 2018).

Phytosterols

The phytosterols content was monitored by following the Liebermann-Burchard and Salkowski tests. The appearance of green colour in the

Liebermann-Burchard's test and brown ring in Salkowski's test confirms the presence of Phytosterols (Table 1). There was no previous findings of phytosterols in watermelon rind.

Sr. No.	Phytochemical Constituents	Observations	Inference					
			Methanolic extracts			Ethanolic extracts		
			M1	M2	M3	E1	E2	E3
1.	**Carbohydrates**							
	Molisch's test	Violet ring	+	+	+	+	+	+
	Fehling's test	Brick red ppt	+	+	+	+	+	+
	Benedict's test	Reddish-brown ppt	+	+	+	+	+	+
2.	**Protein & Amino acids**							
	Ninhydrin test	Purple colour	+	+	+	+	+	+
	Biuret test	Violet colour	+	+	+	+	+	+
	Xanthoproteic test	Yellow colour	+	+	+	+	+	+
3.	**Alkaloids**							
	Mayer's test	Creamy colour	+	+	+	+	+	+
	Wagner's test	Reddish brown ppt	+	+	+	+	+	+
	Hager's test	Yellow ppt	+	+	+	+	+	+
	Dragendorff's test	Red colour ppt	+	+	+	-	-	-
4.	**Flavonoids**							
	Alkaline reagent test	colorless	+	+	+	+	+	+
	Lead-Acetate test	Yellow ppts	+	+	+	+	+	+
5.	**Saponins**							
	Foam test	Persistence foam	+	+	+	+	+	+
6.	**Tannins**							
	Ferric-chloride test	No black, blue or green colour	-	-	-	-	-	-
7.	**Steroids**	Yellow-green ring	+	+	+	+	+	+
8.	**Glycosides**							
	Salkowski's test	Reddish-brown ring	+	+	+	+	+	+
	Keller-Killani test	Brown ring	+	+	+	+	+	+
	Liebermann's test	Green colour	+	+	+	+	+	+
9.	**Phytosterols**							
	Liebermann-Burchard's test	Green colour	+	+	+	+	+	+
	Salkowski's test	Brown ring	+	+	+	+	+	+

Table 1: Qualitative phytochemical screening of Citrullus lanatus rind Antibacterial Activity

The antibacterial activity of n-hexane fractions of different varieties of watermelon rind against gram-positive (Staphylococcus Aureus) and

gram-negative (Escherichia coli) bacterial strains that are presented in Table 2.

Bacteria Type	Pathogenic strains	Diameter of inhibition zones (mm)		
		Charleston gray	Crimson Sweet	Summer Flavor 840
Gram positive	Staphylococcus aureus	0mm	5mm	10mm
Gram negative	Escherichia coli	5mm	0mm	5mm

Table 2. Antibacterial activity of n-hexane extracts

Charleston Gray and Summer Flavor 840 watermelon rind exhibited high antibacterial activity against E.coli with inhibitory zone of 5mm, whereas Crimson Sweet variety showed no activity against the strain. In case of Staphylococcus Aureus, Charleston Gray variety displayed no activity. However, Crimson Sweet variety formed the zone of inhibition of 5mm and Summer Flavor 840 displayed maximum inhibition of 10mm, as shown in table 2. These results were in accordance with work reported by Oluyori et al. 2017. The positive control ciprofloxacin used against both the strains had zone of inhibition of 25mm and against E.coli and 20mm inhibitory zone against Staph aureus. In all experiments the negative control NaCl formed no inhibition zone. Hence, it is concluded from this study that *Citrullus lanatus* have significant antibacterial activity against both Gram positive, Staph aureus, and Gram negative, E.coli bacteria. However, among the three varieties, rind of Summer Flavor 840 watermelon could probably serve as the best antibacterial drug.

Over the past few years, researchers have shown a great interest in creating new antimicrobial drugs to overcome microbial resistance. As the plants produces phytochemicals like flavonoids, alkaloids, tannins, and terpenoids, they are effective against a wide range of microorganism. For this reason plant species have been studied extensively for their antibacterial properties.

Investigations on peels have indicated the existence of essential elements that can be exploited in pharmacological or medicinal applications. Several studies proved that other different parts of watermelon exhibits antibacterial activity. Braide et al. 2012 reported the antibacterial properties of watermelon seeds against different bacteria. Manzoor et al. 2013 analysed that different

concentrations of watermelon juice produces different inhibition zones against Escherichia coli.

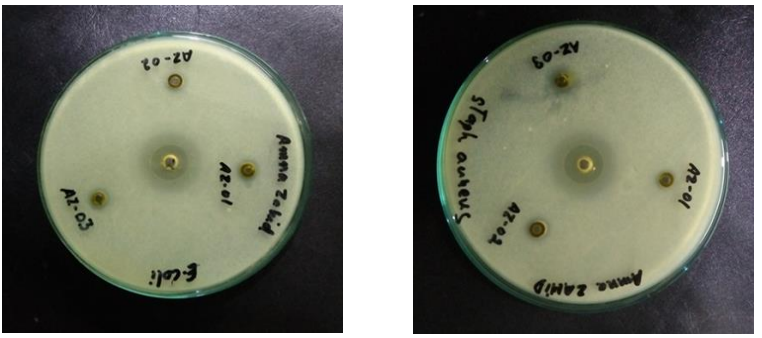

Figure 1: Antibacterial activity against E.coli and Staph aureus

CONCLUSION:

Watermelon (*Citrullus lanatus*) is the top crop across the world, with 6.8% of the world's area dedicated to its production. Because of its thirst-quenching characteristics, it is very popular during hot weather. Watermelon is an extensively produced in Balochistan, having share of 21% in the total production of watermelon in Pakistan. The present study shows that methanolic and ethanolic extracts rind of different varieties (Charleston Gray, Crimson Sweet and Summer Flavor 840) of *Citrullus lanatus* produced in Balochistan contain various phytochemical compounds like carbohydrates, proteins, alkaloids, flavonoids, glycosides, phytosterols, steroids, and saponins. These phytochemicals give plants pharmacological and physiological qualities that can be used to treat a variety of illnesses and could be considered a promising source of therapeutics.

This study reveals that the presence of these secondary metabolites contributes to the antibacterial activity of watermelon rind against common bacteria, Staph aureus and E.coli. Staphylococcus aureus causes fatal infections of lungs, urinary tracts, skin, and soft tissues. E. coli can cause intestinal infection and other diseases. The antibacterial agents present in *Citrullus lanatus* rind can be used to treat such diseases. For all we know, we report the first study on the characterization and antimicrobial properties of rind extracts of different varieties of *Citrullus lanatus* of Balochistan.

FUTURE RECOMMENDATIONS

This research project had shown that the watermelon *(Citrullus lanatus)* rind possesses diverse bioactivities, and hence it can provide preferable advantages in therapies. However, in-depth research and additional measures are hereby recommended in other areas that left to be investigated.

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