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RESEARCH ARTICLE

A study on FTIR, Antimicrobial, Antioxidant and Hypogycaemic effect of *Diospyros kaki* and *Citrullus colocynthis*

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Abstract

Phytochemical, FTIR screening, Hypoglycemic study, antibacterial, antifungal and antioxidant activity of Citrullus colocynthis and D. kaki fruit were studied. The interest of this study is to evaluate traditional herbal usage to treat many diseases around the sultanate of Oman locally. The functional groups that they contain are (O-H) Phenol, (C-H) Alkyl, (N-H) Amine and (C=C) Alkenyl groups corresponds to their biological phytoconstituents responsible for the medicinal properties. The antibacterial and antifungal activities revealed that all parts of tested samples shown good antimicrobial properties. While leaf of C. colocynthis methanolic extract showed highest zone of inhibition (24.33 mm^3) against penicillium. Antioxidant assays were evaluated through DPPH (1, 1- diphenyl-2-picryllhydrazyi) and H2O2 (Hydrogen peroxide scavenging capacity) methods. Peel of D. kaki extract showed higher antioxidant activities than other parts. Antioxidant property of these fruit is due to the presence of significant amount of tannins, phenolic acids and flavonoids on them. A slight change in body weight was observed in all mice compared to the control. All extracts except leaf of C. colocynthis show significant reduction in blood glucose level even more than the positive control which treated with metformin drug. From our investigation of screening different parts of two plants, the results obtained confirm the therapeutic value of both plants used in traditional medicine.

Keywords: FTIR; Antioxidant; Hypoglycaemic; antimicrobial; C. colocynthis; D. kaki

Introduction

In recent times, most plants are medicinally useful in treating disease in the body and in most of cases the antimicrobial efficacy value attributed to some plants is beyond belief. Their use is well established and widely acknowledged to be safe and effective, and may be accepted by national authorities. Active ingredients refer to ingredients of herbal medicines with therapeutic role in treating various human ailments with less side effect. In herbal medicines according to WHO, the preparation of medicines from herbs should be standardized to contain a defined amount of the active ingredients, if adequate analytical methods are available. The most prevalent diseases nowadays in the world which characterized by hyperglycemia that disturb the metabolism of many macromolecules like carbohydrates, lipids and proteins is diabetes mellitus because of deficiency in the action and secretion of insulin [1]. Diabetes mellitus is one of the most five causes of death due to high concentration of sugar in the blood that delay insulin action [2]. Many researches started to find an alternative safe traditional treatment against chronic hyperglycemia. One of the most common traditional therapy in Sultanate of Oman locally to heal diabetes is Citrullus colocynthis. The parts of this plant are used to treat many disease such as: seed for diabetes and leaves for various types of allergies [3]. In addition another medicinal plant used to treat traditionally diabetes is D. kaki (persimmon fruit) by local healers. It has a role in prevention of this disease because it was rich in phytochemicals and antioxidants [4]. Citrullus colocynthis, also known as bitter cucumber, is a desert plant that grows in sandy

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and arid soils. It is also known as bitter apple, and in Oman known as 'Handal'. Its botanical name is Citrullus colocynthis and it belongs to Cucurbitacae family. Citrullus colocynthis has been used effectively in the Indian subcontinent for the treatment of symptoms related to diabetes i.e. hyperglycemia, hyperlipidemia, etc. Citrullus colocynthis (L.) Schrad fruit is an herbal medicine used by traditional herbalists for the treatment of diabetes in Iran [5]. Persimmon (Diospyros kaki) is a soft, very sweet and tasty fruit. It looks like tomatoes. It is the edible fruit of a number of species of trees in the genus Diospyros. Diospyros kaki is evenly high in calories. It gives 70 calories/100 g, but it contains less quantities of lipids. The flesh of persimmon is a very good source of dietary fibres. Diospyros kaki are also a very good source of vitamin C. Regular consumption of persimmon rich in vitamin C helps the body to develop resistance against infectious agents and scavenge harmful proinflammatory free radicals. Many researchers reported that antioxidants and phytonutrients neutralize free radicals that cause degenerative diseases like early ageing, cancer, cataract and macular degeneration. The antioxidant that naturally present in plants found to have anti-infective, anti-inflammatory and anti-haemorrhagic (prevents bleeding from small blood vessels) properties.

Materials and methods

Materials

Persimmon (Diospyros kaki), Citrullus colocynthis, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), Hydrogen peroxide, phosphate buffer, Alloxan monohydrate, Metformin, 8 cages of white albino mice (each cage contain 3 mice), electronic blender, rotary evaporator, autoclave, glucometer, glucometer strips, FTIR and UV Vis Spectrophotometer.

Methods

Preparation of extracts from D. kaki and C. Colocynthis fruits

Flesh and peel of Persimmon (D kaki) and seed, leaf and peel of C colocynthis were collected from the fresh fruits which were earlier collected from market and dried for seven days. Fresh persimmon fruit were divided into two parts (flesh and peel) and C colocynthis were divided into three parts (seed, leaf and peel). Seeds of C colocynthis were washed with tap water and salt to remove the bitter taste. The collected sample parts were oven dried at 60°C for 24 hrs and grounded into fine powder in electronic blender. Each 20g of dried parts of both samples was extracted through maceration with 200ml of two different solvents (methanol and ethanol). Under constant pressure and temperature the filtrate containing solvents were evaporated in rotary evaporator at 64.7°C (methanolic extract) and 78.37 °C(ethanolic extract). All sample crude extracts were placed in a glass bottle and stored at 4°C until further usage.

Phytochemical screening

Major bioactive phytoconstituents such as: tannins, alkaloids, terpenes, saponins, cardiac glycosides, flavonoids, phenolic and carbohydrates were screened in D. kaki and C. Colocynthis extracts according to [6]. Samples were prepared by dissolving 1 gram of each dried crude extract in 50ml of distilled water. The extractive values are calculated according to standard protocols [7].

Fourier Transform Infrared Spectrophotometer (FTIR) analysis

Fourier Transform Infrared Spectrophotometer (FTIR) is one of the analytical tools for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 $^{cm-1}$ with a resolution of 4 $^{cm-1}$.

Antioxidant study

DPPH radical scavenging activity (RSA) Different concentrations (25 μl, 50 μl, 75μl and 100 μl) of each sample were added to 2ml of DPPH in a test tube. After 30 minutes of incubation, the absorbance was measured at 517 nm against blank. The control standard is prepared by taking 2 ml of DPPH without extract [8]. The percentage inhibition of DPPH radical scavenging activity (RSA) was calculated by the following formula:

DPPH RSA%= [1 – (Absorbance of sample / Absorbance of control) $\times$ 100[9]

H_2O_2 scavenging capacity

H_2O_2 scavenging capacity of each extract was determined according to [9]. Different concentrations (50 μl, 100 μl, 150 μl and 200 μl) of each sample were added to a 0.6 ml of hydrogen peroxide solution (40 mM). Absorbance of hydrogen peroxide at 230 nm was determined against blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of H_2O_2 of both sample extracts were calculated by following formula:

H_2O_2 scavenging capacity =[(Control OD – Sample OD) / Control OD] $\times$ 100

Antimicrobial study

Test organisms: The antibacterial and antifungal activities of extracts of D. kaki and Citrullus colocynthis were tested against three Gram-positive bacteria—Staphylococcus aureus, bacillus circulens, micrococcus lutein, two Gram-negative bacteria—Escherichia coli, salmonella enteric and two fungal strains penicillium chrysogenum and Rhizopus stolonifer. The overnight stains were collected from microbiology lab, Higher College of Technology, Alkhuwair, Muscat, and Sultanate of Oman. Fungal suspension is prepared from respective stock by inoculating 2-3 loops of fungal spores into sterile distilled water.

Test medium: In vitro investigation of antimicrobial study was carried out using nutrient agar medium and PDA medium are used for antifungal study.

In-vitro growth inhibition study: 100 μl from each broth was inoculated on agar plate of respective microorganism suspension by using the micropipette. The L- shape spreader was used to spread the microorganism in the agar plate. Three replicates were used for each species. Then, cultures were incubated in the incubator for 24 to 48 hours at 37^C and fungi at 25°C in an up-side down position. The mean values of three trials zone of inhibition and standard deviations were calculated.

Hypoglycemic effect of D. kaki and C. colocynthis fruit extracts

2ml of each methanol and ethanol extract of different parts of D. kaki and C. Colocynthis was diluted with 20ml of sterile distilled water and filtered using 0.5 U Millipore. Only male mice were selected and 8 cages were prepared. 3 mice were caged as each individual group. Cages were labeled as follows: cage 1 for ethanol extract of the flesh of D. kaki, cage 2 for ethanol extract of the peel of D. kaki, cage 3 for ethanol extract of the peel of C. colocynthis, cage 4 for ethanol extract of the seed of C. colocynthis, cage 5 for ethanol extract of the leaf of C. colocynthis, cage 6 for normal control, cage 7 for positive control where mice were given alloxan and treated with medicine and cage 8 for negative control where mice were given alloxan without treatment. The mice weight was recorded and the blood sugar was measured followed by diabetes was induced with alloxan (0.1% Alloxan monohydrate prepared in normal saline) at 150mg/Kg body weight except normal control cage. Dosage of extract was administered orally 1 ml per day and continued for 21day. Metformin HcL 850 mg was given to mice as a positive control. After 21 days, the weight of all mice was measured and recorded. The blood glucose of all mice was taken by intraperitoneally.

Result and Discussion

Phytochemical analysis of plants extract

Phytochemicals are secondary compounds that have ability to prevent diseases. The results of phytochemical screening are presented in table 1. Both methanol and ethanol solvents were shown almost similar results.

Ethanolic and methanolic extracts of flesh and peel of D. kaki contain high amounts of saponins (+++) and carbohydrate (+++), moderate amounts of tannins (++), less amount of phenolic (+) and flavonoids (+) and they were absent from alkaloids (-), cardiac glycoside (-) and terpenes (-). Tannins are one of essential bioactive molecule found in the flesh of persimmon fruit. C. colocynthis contains high amount of tannins (+++), phenolics (+++) and flavonoids (+++) in its leaf part. Methanolic extract of Seed of C. colocynthis have high amount of saponins (+++). Peel and seed contain moderate amount of tannins (++), saponins (++), phenolics (++) and carbohydrate (++). All parts of C. colocynthis contain less amount of alkaloids (+). The analysis of C. colocynthis extract document the presence of flavonoids and tannins which are important bioactive secondary compounds responsible might be responsible for antioxidant and various biological activity. There are high quantities of bioactive substances can be found in fresh persimmon which are evaluated to have antioxidant efficiency [10].

FTIR analysis

This analysis uses the infrared light to analyse samples for detecting the range of functional groups of them. The functional groups with their probable phytochemicals of different parts of D. kaki and C. colocynthis are shown in table 2. Peel and Leaf of C. colocynthis with ethanol have more functional groups. The functional groups that they contain are N-H stretching vibrations of amine which absorb in a range 3500 – 3300 (cm-1), C-H stretching vibrations of alkyl which absorb in a range 2950 - 2850 (cm-1) and C=C stretching vibrations of alkyl which absorb in a range 1680 - 1620 (cm-1). Methanol and ethanol extract of flesh and peel of D. kaki show that both of them contain Phenol (O-H), which absorb in a range of 3550 - 3230 cm-1.From the results obtained through FTIR , it is significantly clear that C.colocynthis in both the extracted solvents showed the presence of more active functional groups. These functional groups intern responsible for the presence of active ingredients like phytoconstituents which are the key factor of any medicinal plant for their efficient medicinal property. The higher antimicrobial and hypoglycaemic potency of C.colocynthis than D Kaki studied in the later sessions is also evidenced due to the more active functional groups. The phytochemical screening studied earlier session also revealed that C.colocynthis ethanolic extract showed better phy-

Table 1 Phytochemical screening offlesh and peel of Persimmon (D kaki) and C colocynthis extracts.

Parts of D. kaki				Parts of C. colocynthis						Test
Peel		Flesh		Leaf		Seed		Peel		/
E	M	E	M	E	M	E	M	E	M	sample
+ +	+ +	+ +	+ +	+++	+++	++	++	++	++	Tannins
-	-	-	-	+	+	+	+	+	+	Alkaloids
+++	+++	+++	++	++	++	++	+++	++	++	Saponins
-	-	-	-	-	-	-	-	-	-	Cardiac glycoside
+	+	+	+	+++	+++	++	++	++	++	Phenolic
+	+	+	+	+++	+++	++	++	++	++	Flavonoids
-	-	-	-	-	-	-	-	-	-	Terpenes
+++	+++	+++	+++	++	++	+ +	+ +	++	++	Carbohydrate

Note: All the values expressed in the table are the mean of 3 replicates. +++: strong presence, ++: moderate, +: less and - : absence.

Table 2 FT-IR analysis of different parts of D. kaki and C. colocynthis fruits extracts with ethanol and methanol solvents.

Solvent	Plants	Parts	Peak value or wavelength cm $^{-1}$	Predicted functional group	Probable phytochemicals
Methanol	D.kaki	Flesh	3286.74	(O-H) Phenol	Alkaloids, flavonoids, poly phenol, carboxylic acid and tannins.
			2840.08	(C-H) Alkyl	
		Peel	3286.08	(O-H) Phenol	
			1640.25	(C=O) Amide	
	C.colocynthis	Leaf	3288.18	(O-H) Phenol	
			2839.97	(C-H) Alkyl	
		Seed	3281.90	(O-H) Phenol	
			2840.95	(C-H) Alkyl	
		Peel	3354.27	(N-H) Amine	
			1640.24	(C=C) Alkenyl	
Ethanol	D.kaki	Flesh	3277.47	(O-H) Phenol	
		Peel	3277.65	(O-H) Phenol	
			1640.08	(C=C) Alkenyl	
	C.colocynthis	Leaf	3320.20	(N-H) Amine	
			2980.38	(C-H) Alkyl	
			1640.31	(C=C) Alkenyl	
		Seed	3316.26	(N-H) Amine	
			1640.12	(C=C) Alkenyl	
		Peel	3331.79	(N-H) Amine	
			2981.32	(C-H) Alkyl	
			1640.14	(C=C) Alkenyl	

Note: Plants which analysed are Diospyros kaki (Common name = persimmon fruit) and C colocynthis (Common name = bitter melon).

toconstituents like phenols, flavonoids saponins etc. at higher concentration than D Kaki. Presence of phenols and flavonoids contribute the medicinal plants as chief source of antioxidants.

DPPH radical scavenging activity (RSA)

The ability of parts studied in D. kaki and C. colocynthis to check anti-oxidative activity were discussed according to the % inhibition at different concentrations ranging from 25-100 ul/mg. As shown in Table 3.3, the extract from peel of D. kaki has the strongest DPPH RSA % (95.57 % inhibition of DPPH radical at 75ul concentration) in ethanol extract compared to the flesh part (92.92 % inhibition at 75ul concentration). Similarly, [11] demonstrated that D. kaki peel with ethanol extract showed stronger DPPH RSA than flesh part. On other hand, the extract from leaf of c. colocynthis has lowest value of DPPH RSA% shown 85.9 % antioxidant at 50μl .

As shown in Table 3.3, the methanol extract of flesh D.kaki has highest DPPH RSA % shown 95.57% antioxidant at 50 μl/mg. While, the extract from peel of C.colocynthis has the stronger DPPH RSA% in all 75μl concentration than other parts. [12] Confirmation that C. Colocynthis with methanol was screened to evaluate its free radical scavenging effect. It has highest antioxidant and free radical scavenging. The lowest values were noted in seed of C. colocynthis fruit methanol compared to other parts. D. kaki contains many compounds which work as protective scavengers against oxygen derived free radicals and reactive oxygen species that play a role in aging and various disease processes such as catechins,lycopene,

Table 3 DPPH RSA of different concentrations of extracts from different parts of plants used in the study by solvents.

Sample	DPPH RSA 100% Ethanol(ul/mg)				Methanol(ul/mg)			
	25	**50**	**75**	**100**	**25**	**50**	**75**	**100**
D. kaki (Flesh)	92.47	92.62	**92.92**	91.74	**95.57**	91.74	91.44	90.41
D. kaki (Peel)	90.5	92.77	**95.57**	91.00	**93.21**	91.74	88.64	87.46
C. colocynthis (Peel)	91.74	92.92	**93.21**	90.70	**95.42**	94.54	94.24	90.56
C. colocynthis (Seed)	87.31	88.05	**89.08**	88.79	78.90	78.61	**89.97**	88.79
C. colocynthis (Leaf)	86.13	85.98	**92.03**	88.79	88.34	93.95	88.20	**92.62**

Note: All the values expressed in the table are the mean of inhibition percentage of tri plicates and 25 μl, 50 μl, 75 μl and 100 μl are the different concentrations taken from fruit extracts.

beta-carotene, cryptoxanthin lutein and zeaxanthin and gallocatechins.

Hydrogen peroxide scavenging capacity

The extract from flesh of D.kaki has the strongest H_2O_2 RSA % (89.8 % inhibition at 100 μl concentration) in methanol extract .While ,the peel of C.colocynthis has lowest H_2O_2% (22.4% inhibition at 150 μconcentration) compared to other parts. This study was showed that D.kaki was the more effective source of natural antioxidants.DNA can be damaged by hydroxyl free radical generated by H_2O_2, and this oxidative damage can be inhibited by peel of persimmon fruit [13]. The ability of parts studied of D. kaki and C. colocynthis to have anti -oxidative activity were discussed according to the % inhibition in different concentration. Our results show that extract of leaf from C.colocythis has strongest H_2O_2% (91.6% inhibition at 25ul concentration) compared to other parts. The ability of D. kaki parts to inhibit hydrogen peroxide free radicals is range from 30% to 63% inhibition. The ability of C. colocynthis to provide antioxidant activity is due to phenolics [12]. Phenolic compounds present in persimmon fruit can provide hydrogen from OH groups in phenolic substances to remove the OH• radical produced by H_2O_2 [13]. D. kaki is a rich source of many powerful antioxidants such as polyphenols, carotenoids, vitamin C and tannin contents which help to inhibit the damages produced by free radicals in body tissues [14].

Antimicrobial study

Antibacterial study

Table 5 Shows Inhibition zone of different types of bacteria against different parts of D.kaki and C colocynthis fruits by using two different solvent extraction (methanol and ethanol). The results showed as remarkable inhibition of the bacterial growth was shown against the tested bacterial strains are due to the presence of effective biocides. C colocynthis is a good source for treatment of various human diseases, because it contains significant amount of secondary metabolites such as saponins, tannins, alkaloids and flavonoids. Tannins hinder the availability of essential proteins which are important for the growth of microorganisms. Flavonoids present in C colocynthis thoroughly responsible as inhibitor of microbial growth even stains are resistant to antibiotics [15]. It was reported that the leaf extracts from C. colocynthis showed greater inhibitory activity against gram positive and gram negative bacteria.The extract from D. kaki is an effective antibacterial for different pathogens like E. coli and S. aureus due to the presence of polyphenol compound on it is parts [16].

Inhibition zone of different parts of D.kaki and C colocynthis fruits against E.coli. Clearly seen from above figure that the Leaf of C. colocynthis had highest zone of inhibition in both ethanol (16 mm) and methanol (14.6mm).Peel of persimmon has lowest zone of inhibition(6.33mm) in methanol extraction while seed of C. colocynthis has lowest zone of inhibition (6mm) in ethanol extraction. Table 5 Shows inhibition zone of shows Inhibition zone of different parts of D.kaki and C colocynthis fruits against M lutein. The Seed of C.colocynthis and flesh of D.kaki were observed that they have almost similar zone of inhibition in methanol extraction (18mm and 18.3mm). On other hand, Peel of C.colocynthis and Leaf of C.colocynthis were observed that they have similar zone of inhibition in ethanol extraction (13.6mm and 13.3mm) while peel of D. kaki has lowest zone of inhibition in both extraction. Inhibition zone of different parts of fruits studied against S.aureus. The Flesh of D. kaki had highest zone of inhibition in ethanol (23 mm), while leaf of C.colocynthis has highest zone of inhibition (19.3mm) in methanol extraction. Peel of persimmon has lowest zone of inhibition (6mm) in ethanol extraction. On other hand, Seed of C.colocynthis has lowest zone of inhibition (6mm) in methanol extraction. Zone of inhibition against different parts of D.kaki and C colocynthis fruits against S enterica. The Peel of D. kaki had highest zone of inhibition in both ethanol (20.6 mm) and methanol (21mm). Flesh of D.kaki has lowest zone of inhibition in both extraction (0mm). Inhibition zone of different parts of D.kaki and C colocynthis fruits against B circlens. The Flesh and peel of D. kaki were observed that they have similar zone of inhibition in both methanol extraction (25.6mm and 25 mm) and

Table 4 Hydrogen peroxide RSA of differentconcentrations of extracts

	H_2O_2 RSA %							
	Ethanol				Methanol			
	50	100	150	200	50	100	150	200
D. kaki (Flesh)	57.1	89.8	76.9	63.7	78.1	49.7	63.7	45.2
D. kaki (Peel)	76.2	81.8	69.2	45.2	65.6	42.4	57.1	30.6
C. colocynthis (Peel)	84.0	82.9	54.3	51.6	82.2	68.4	81.09	37.6
C. colocynthis (Leaf)	80.3	50.4	22.4	61.7	91.6	30.9	37.6	31.3
C. colocynthis (seed)	88.3	81.4	73.09	57.1	77.5	74.6	75.6	37.6

Note :All the values expressed in the table are the mean of inhibition percentage of 2 replicates and 50μl, 100 μl, 150 μl and 200 μl are the different concentrations taken from fruit extracts.

Table 5 Antibacterial activity of different parts of D kaki and C colocynthis against different types of bacteria.

B.circulens	S. enterica	S.aureus	M. lutein	E.coli	Solvent	Parts	Plants
31±0.57	31.6±0.66	32.3 ±2.027	40 ± 0	26.6 ± 0	C		
0±0	17.6± 0.33	16.3±1.855	14±7.09	14 ± 0.577	M	Peel	
22.6± 0.66	15±0.57	13.6±0.333	13.6± 6.88	12.6± 0.66	E		
33.6±0.33	34.6±0.33	29.3±1.201	40±0	26.6 ± 0.88	C		
15.6±7.96	16.3±0.66	12.6±6.359	18± 1.15	9 ± 4.58	M	Seed	C. colocynthis
13.6±6.83	19±1	14± 0.88	21±4.04	6 ± 6	E		
32±1.15	31.6±0.88	31.3±0.666	40±0	25.6 ± 0.66	C		
20 .3±0.33	20.3±0.33	19.3 ±1.763	14 ±2.51	14.3±1.855	M	Leaf	
16.6±1.20	12.3± 6.17	21.6± 2.185	13.3± 1.33	16±1	E		
34.6±0.33	30.3± 0.33	24±0.577	40±0	26.3±0.881	C		
25.6±4.33	0±0	16 ±1.527	18.3±9.20	11.6± 1.201	M	Flesh	
27.6±1.20	0±0	23± 1.527	6±6	9± 4.50	E		D. kaki
34 ±0	31.6± 0.881	30.3±1.45	40±0	26 ±1	C		
25±5	21.3± 0.88	14.6±1.763	4 ± 4	6.33± 6.33	M	Peel	
27.6±0.33	20.6±0.66	11±1.855	3.33±3.33	7±7	E		

Note: All measurements in all above tables were done in three replicates, and all values are means ± standard error. Where, C=control, M= methanol extract, and E=ethanol extract.

they are sharing same zone of inhibition in ethanol (27.6mm). On other hand, Peel of C.colocynthis has lowest zone of inhibition in methanol (0mm). Seed of C.colocynthis has lowest zone of inhibition in ethanol extraction (13.6mm).

Antifungal study

Antifungal study was carried out to investigate the antifungal potential of peel, seed, leaf of C. colocynthis, flesh and peel of D.kaki against pencillium and Rhizopus. Table 6 shows fungus growth and zone of inhibition of P chrysogenum and R stolonifer with the different parts of plants studied.

The effect of extracts against P chrysogenum is as follows: leaf of C. colocynthis had the highest zone of inhibition in methanol extraction (24.33 mm^3) , and it has almost no much different compared to the zone of inhibition of artificial antifungal used as a control. Peel of C. colocynthis and flesh of D. kaki in methanol extraction have the same zone of inhibition (20.33 mm^3). Seed of C. colocynthis had the lowest zone of inhibition in methanol extraction. On the other hand, peel of D.kaki had the highest zone of inhibition in ethanol extraction while peel of C. colocynthis had the lowest zone of inhibition. Whereas, the ability of these extracts to have antifungal potential against R stolonifer under the different parts of plants studied is as follows : Peel (23.33) and seed (23.66) of C. colocynthis had the highest zone of inhibition in methanol extraction. Flesh of D. kaki had the lowest zone of inhibition in methanol extraction. On the other hand, peel of C. colocynthis had the highest zone of inhibition in ethanol extraction while flesh of D. kaki had the lowest. We can conclude, that these plants secrete some of ingredients which provide them with potential to act as a fungicide against pathogenic fungus.

D.kaki have different types of phenolic compounds such as m-gallate, gallic acid luteolin ,quercetin , and myricetin responsible for antibacterial and antifungal activity. The phytoconstituents of D Kaki seed showed functional groups like: polyphenols, steroids and alkaloids which are the potent antifungal agents [17].

Hypoglycaemic effect of D. kaki and C. colocynthis fruits extracts:

Diabetes is a metabolic disease which grows very fast in the world. People demand for a safe treatment to this prevalent dis-

Table 6 Antifungal activity of D kaki and C colocynthis against P chrysogenum and R stolonifer.

R. stolonifer	P. chrysogenum	Solvent	Part	Fruit
23±0.57	26.33± 0.66	C		
23.33± 0.66	20.33± 0.33	M	Peel	
18.33± 3.33	18±0.57	E		
25.33±1.33	26.66±0.33	C		C. colocynthis
23.66±2.33	18.33±0.33	M	Seed	
14.3± 0.33	20±0	E		
22±2	27.66±0.33	C		
16.33±1.33	24.33±0.66	M	Leaf	
16± 1.52	20.66±0.33	E		
22.33± 0.88	26.33± 0.33	C		
15± 1.52	20.33± 0.33	M	Flesh	
14± 0	23.33± 0.33	E		D. kaki
22± 0.57	30.66±0.66	C		
16.6± 0.88	19± 0.57	M	Peel	
15.66± 0.33	24.33± 0.33	E		

Note: All measurements were done in three replicates, and all values are means ± standard error. Where, C=control, M= methanol extract, and E=ethanol extract

ease, so many researchers reported about the use of traditional medicinal plants as a new natural key which have anti-diabetic property [18]. Flesh and peel of Persimmon (D kaki) and seed, leaf and peel of C colocynthis ethanolic extracts were experimented with alloxan induced mice to evaluate their anti-diabetic potential and weight change. A slight change in body weight was observed in all mice compared to the control. Only 0.5 g weight gain was observed in diabetic control mice. The most reduction in body weight was noticed in the flesh of D. kaki extract (23.9g to 20.15g), followed by the peel of D. kaki (25.4g to 24.4g), whereas a fractional increase in body weight was seen in peel and leaf of C. colocynthis. No change in weight with seed extract of C. colocynthis was observed. Weight gain is significant risk factor for diabetes, hyperglycemia increases proportionally with greater body weight was reported in many clinical trials [19]. Deficiency in insulin can cause the degradation of structural proteins which involve for the body weight, and the ability of D. kaki and C. colocynthis to prevent maximum loss of body weight is because their potential capacity to protect from protein degradation which provide hypoglycemic activity against alloxan induced mice [20].

Alloxan induced diabetes in mice resulted in significant increase in blood glucose levels. After oral administration of the ethanolic extracts of D. kaki and C. colocynthis (1ml was given by syringe for each mice) to diabetic mice up to 21 days, a clear decrease in plasma glucose was observed compared to normal control and positive control which was treated with artificial drug. The peel of C. colocynthis extract showed the significant reduction in blood glucose level (Overall Hypoglycaemic = 92(mg/dl)↓) followed by the peel of D. kaki extract (46.4(mg/dl)↓), whereas the leaf of C. colocynthis showed fractional increase in blood glucose level (-10.3↑).

All extracts except leaf of C. colocynthis showed significant reduction in blood glucose level even more than the positive control which treated with metformin drug. C. colocynthis shown hypoglycaemic effect in diabetic mice. Peel of persimmon (D. kaki) is rich of antioxidants such as vitamin C and phenolics and higher levels of phytoconstituents which are responsible as anti-diabetic agents by enhancing either production or encouraging cells to uptake blood glucose. The hypoglycemic role and mechanism of these natural fruits is may be due to inhibitory effect on pancreas alpha amylase [17]. A great attention must be taken on these traditional plants which really have natural effective compounds to start a new branch for novel medicinal agents to treat various death causative disorders and diseases.

Clearly seen from above figure 3.15 that the peel of C. colocynthis has the higher hypoglycaemic activity followed by the peel of D. kaki extract. Aqueous extract obtained from C. colocynthis fruit was studied in alloxan induced rabbits, their results indicate the presence of saponines and glycosidic bioactive substances in C. colocynthis help in lowering blood glucose level [21]. The reports explain the peel of D. kaki as a beneficial source of antioxidants which helps in lowering the oxidative stress increased by hyperglycemia in the diabetic condition [22]. [23]^{Demo}nstrated that flavonoids are bioactive compounds present naturally in many plant material; it reported to have effect on glucose metabolism. So, as C. colocynthis and D. kaki fruits contain significant amount of flavonoids; they might be responsible to show hypoglycemic action. The study also supports that enzymes present in D. kaki fruit namely α-glycosidase and α-amylase are responsible to digest carbohy-

Table 7 Effect of ethanolic extract of different parts of D. kaki and C. colocynthis on the weight of experimental mice.

p-value	t-value	df	Change in body weight (g) after 21 days	Final body weight (g)	Initial body weight (g)	Name of cage
0.133	1.51	2	-3.75	20.15±6.8	23.9±0.1	D.kaki (Flesh)
0.33	0.49	2	-1	24.4±0.1	25.4±2.65	D.kaki (Peel)
0.171	-1.23	2	1.75	27.7±0.4	26.13±1.2	C. colocynthis (peel)
0.5	0	2	0	22.5±3	22.5±1.9	C. colocynthis (seed)
0.21	-1	2	0.53	22.3±2.2	21.8±21.8	C.colocynthis (Leaf)
0.216	-0.97	2	2.4	21.6±1.5	19.2±1.2	Normal control
0.21	0.98	2	0.5	28.5±1.5	28 ±0.5	Diabetic control
0.12	-1.6	2	0.8	23.5±2.2	22.7±1.83	Control with drug

Note: Values are mean of 3 mice each ± SD.

Table 8 Effect of ethanolic extract of different parts of D. kaki and C. colocynthis on the blood glucose (mg/dl) in experimented mice.

Name of cage	Blood glucose after alloxan	Blood glucose after treatment	Over all Hypoglycemic (mg/dl)	T-test	Df	P-value
D.kaki (Flesh)	146±4	108.5±3.5	38↓	1.99	2	0.091
D.kaki (Peel)	176±34.3	129.6±18.8	46.4↓	2.97	2	0.048
C. colocynthis (Peel)	292±106	200±39.7	92↓	0.69	2	0.279
C. colocynthis (seed)	192±26.7	161±2.8	31↓	8.188	2	0.007
C.colocynthis (Leaf)	162.3±4.9	172.6±8	10.3↑	-1	2	0.007
Normal control	129.3±23.9	133.3±4	4↑	0.211	2	0.211
Diabetic control	122±10.5	157.5±24.5	35.5↑	0,37	2	0.377
Control with drug	160±7.2	132.3±15.3	27.7↓	1.46	2	0.140

Note: All the values expressed in the table are mean values of 3 sample and are mg/dl± standard deviation; with paired t test analysis.

drates into monosaccharide which may possessed hypoglycemic effect. It was reported that the reduction in serum glucose in treated mice is due to significant utilization of glucose [24].

Conclusion

This study was the first attempt to evaluate the biological activities sharing between C. Colocynthis and D. Kaki fruits. The health benefit properties of these fruits might be related with the different phytochemicals, antioxidants and functional groups present on them. Most the extracts have significant antibacterial activity on most of the bacteria tested in this study. It can be concluded that oral administration of D. Kaki and C. Colocynthis fruits had the ability to reduce the serum glucose in diabetic mice. A great attention should be taken on these fruits to test the different natural substances present. This research would open unique approach to extract new therapeutic medicines from natural way to cure different diseases with less or no side effects.

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References

[1] Sobia K, A JM, Rehmatullah AMS, Hina Q, Iram G, Pervaiz B, et al. Assessments of Phytochemicals and Hypoglycemic Activity of Leaves of Carica papaya in Diabetic Mice. International Journal of Pharmaceutica Science and Research. 2016;7(9):3658–3665.

[2] Dallak M, Bin-Jaliah I; 2010.

[3] Al-Ghaithi F, El-Ridi MR, Adeghate E, Amiri MH. Biochemical effects of Citrullus colocynthis in normal and diabetic rats. Molecular and Cellular Biochemistry. 2004;261(1):143–149. Available from: https://dx.doi.org/10.1023/b:mcbi.0000028749.63101.cc.

[4] Butt MS, Sultan MT, Aziz M, Naz A, Ahmed W, Kumar N, et al. Persimmon (Diospyros kaki) fruit: hidden phytochemicals and health claims. EXCLI Journal. 2015;14:542–561.

[5] Huseini HF, Darvishzadeh F, Heshmat R, Jafariazar Z, Raza M, Larijani B. The clinical investigation ofcitrullus colocynthis(L.) schrad fruit in treatment of Type II diabetic patients: a randomized, double blind, placebo-controlled clinical trial. Phytotherapy Research. 2009;23(8):1186–1189. Available from: https://dx.doi.org/10.1002/ptr.2754.

[6] Wani BA, Ramamoorthy D, Ganai BA. Preliminary Phytochemical Screening and Evaluation of Analgesic Activity of Methanolic Extract of Roots of GentianakurrooRoyle in Experimental Animal Models. International Journal of Pharmacy and Pharmaceutical Sciences. 2011;3:975–1491.

[7] Pharmacopoeia E. General Organization for Governmental Printing Office. Cairo. 1984;.

[8] Tugba O, Ece S, Zeynep T, Dilara NE. Phenolic Contents and Antioxidant Activities of Persimmon and Red Beet Jams Produced by Sucrose Impregnation. Food Science and Technology. 2015;3(1):1–8.

[9] Keser S, Celik S, Turkoglu S, Turkoglu YO, I. Hydrogen Peroxide Radical Scavenging and Total Antioxidant Activity of Hawthorn. Chemistry Journal. 2012;02:9–12.

[10] Jung ST, Park YS, Zachwieja Z, Folta M, Barton H, Piotrowicz J, et al. Some essential phytochemicals and the antioxidant potential in fresh and dried persimmon. International Journal of Food Sciences and Nutrition. 2005;56(2):105–113. Available from: https://dx.doi.org/10.1080/09637480500081571.

[11] Jang IC, Bae JEK, S M, J LH, I JG, E P, et al. Antioxidant and Antigenotoxic Activities of Different Parts of Persimmon (Diospyros kaki cv. Fuyu) Fruit Journal of Medical Plants Research. 2010;4(2):155–160.

[12] Kumar S, Kumar D, Jusha M, Saroha K, Singh N, Vashishta B. Antioxidant and free radical scavenging potential of Citrullus colocynthis (L.) Schrad. methanolic fruit extract. Acta Pharmaceutica. 2008;58(2):215–220. Available from: https://dx.doi.org/10.2478/v10007-008-0008-1.

[13] Kim SY, Jeong SM, Kim SJ, Jeon KI, Park E, Park HR. Effect of heat treatment on the antioxidative and antigenotoxic activity of extracts from persimmon (Diospyros kaki L.) peel. Biosci Biotechnol Biochem. 2006;70:999–1002.

[14] George AP, Redpath S. Health and medicinal benefits of persimmon fruit: a review. Advances in Horticultural Science. 2008;22(4):244–249.

[15] Bnyan I, Hasan H, Ewadh M. Antibacterial Activity of Citrullus Colocynthis against different types of bacteria. Advances in Life Science andTechnology. 2013;7:48–51.

[16] HARA-KUDO Y, KOBAYASHI A, SUGITA-KONISHI Y, KONDO K. Antibacterial Activity of Plants Used in Cooking for Aroma and Taste. Journal of Food Protection. 2004;67(12):2820–2824. Available from: https://dx.doi.org/10.4315/0362-028x-67.12.2820.

[17] Butt MS, Sultan MT, Aziz M, Naz A, Ahmed W, Kumar N, et al. Persimmon (Diospyros kaki) fruit: hidden phytochemicals and health claims. EXCLI Journal. 2015;14:542–561.

[18] Sattar NA, Hussain F, Iqbal T, Sheikh MA. Determination of in vitro antidiabetic effects of Zingiber officinale Roscoe. Brazilian Journal of Pharmaceutical Sciences. 2012;48(4):601–607. Available from: https://dx.doi.org/10.1590/s1984-82502012000400003.

[19] Colditz GA, Mbbs, Drph, Walter C, Willett, Andrea M, et al. Weight Gain as a Risk Factor for Clinical Diabetes Mellitus in Women. Annals of internal medicine. 2004;.

[20] Gurudeeban S and Ramanathan T (2010). Antidiabetic Effect of Citrulluscolocynthis in Alloxan-Induced Diabetic Rats. Inventi Journals, 1, Issue 1.

[21] Abdel-Hassan IA, Abdel-Barry JA, Mohammeda ST. The hypoglycaemic and antihyperglycaemic effect of Citrullus colocynthis fruit aqueous extract in normal and alloxan diabetic rabbits. Journal of Ethnopharmacology. 2000;71(1-2):325–330. Available from: https://dx.doi.org/10.1016/s0378-8741(99)00215-9.

[22] Yokozawa T, Kim YA, Kim HY, Lee YA, ichiro Nonaka G. Protective effect of persimmon peel polyphenol against high glucose-induced oxidative stress in LLC-PK1 cells. Food and Chemical Toxicology. 2007;45(10):1979–1987. Available from: https://dx.doi.org/10.1016/j.fct.2007.04.018.

[23] Bae UJ, H PS, Y JS, B P, S C. Hypoglycemic effects of aqueous persimmon leaf extract in a murine model of diabetes. Molecular Medicine Reports. 2015;12(2):2547–2554.

[24] Reddy SH, Al-Neeri IS, Al-Issaei HK, Al-Jabri SM. Effect of Selective Medicinal Plant Extract on Blood Glucose, Sperm Shape and Various Physiological Parameters. American Journal of Plant Sciences. 2015;06(08):1109–1115. Available from: https://dx.doi.org/10.4236/ajps.2015.68115.