Comparative Assessment of Biological Properties of *Docyniaindica*Decne.Peel and Pulp (Assam apple)

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ABSTRACT

This research paper aimed to investigate the total phenol content, antimicrobial activities and antioxidant activities of *Docyniaindica*Decne. (Assam apple). Phytochemicals of peel and pulp of selected sample revealed the presence of alkaloids, carbohydrates, saponins, glycosides, reducing sugars, flavonoids, tannins, α- amino acids, starch and phenolic compounds. Functional groups in pulp and peel of *D.indica*were examined by FT-IR spectra which showed the present of phenolic compounds. Total Phenol Content (TPC) of ethanol and water extracts of peel were 34.15 μg and 25.39 μg and ethanol and water extracts of pulp were 44.35 μg and 38.71μg respectively. TPC of pulp extracts were higher than peel extracts. In addition, antimicrobial activities of five solvent extracts were carried out using agar well diffusion method. In the determination of antioxidant activities, the IC_{50} value of ethanol and water extracts of pulp and peel were found to be 3.82μgmL^{-1}, 1.59μgmL^{-1}, 0.86 μgmL^{-1} and 15.14μgmL^{-1} respectively. The IC_{50} of ascorbic acid is 1.17μgmL^{-1}. The pulp ethanol extract was the most effective in other extracts and standard ascorbic acid. The potential to develop Assam apple extract as an antioxidant drug is a thrust area for future research in drug designing industry.

Keywords: *Docyniaindica*Decne, Phytochemical investigation, FTIR, Antioxidant Activity, TPC

Introduction

Plants are used in folk medicine to treat different illness of human beings. A number of natural compounds extracted from plants offer alternative treatment options that are safe and effective..

Assam apple fruits are usually harvested around September to October. It is distributed in eastern Himalayas, South-east Asia, from Vietnam through India and north into central China, Bhutan, India, Myanmar (Kachin state, and Shan state).Ittypically occurs at elevation above 1000 m and is most common at 1500-1700 m. It has been used traditionally as a supplement remedies, stimulating digestion, appetite, bloating treatment, and heartburn. Assam applecan also be combined with other herbals in treatment of tonic spleen and stimulating digestion. According to the local knowledge, Assam applecontains high nutrient value and biological substances, which are necessary for the human body.

Botanical Description of *Docyniaindica*Decne.

Family name : Rosaceae
Scientific name : *Docyniaindica*Decne.
Myanmar name : Pin-seinn-thee
Common name : Assam apple
Part used : Peel and pulp of Fruits
Medicinal uses : infectious diseases, digestive, hypoglycemia and total cholesterol, triglycerol, LDL reducing effect and anti-obesity, appetite, Bloating, heart burn, syrup, alcohol, wine, and Vinegar.

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Figure1.Photograph of tree, fruits and flowersof *Docyniaindica*Decne.

Phytochemicals

Phytochemicals are biologically active; naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients.

Antioxidants

Antioxidants are chemicals that interact and neutralize with free radicals, thus preventing them from causing damage. Antioxidants are also known as "free radical scavengers". The body makes some antioxidants it uses to neutralize free radicals.

Antioxidant activity by DPPH radical scavenging method

Antioxidants play an important role as health protecting factor. A rapid simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) which is widely used to test the ability of compound to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity.

Materials and Methods

Plant material

The peel and pulp of *Docyniaindica*Decnewere collected fromLoilem, Southern Shan State, during June,2017.The sample was cleaned by washing with distilled water and then dried at room temperature.The dried sample was grounded into purely fine powder by using an electric blender. It was stored in a well-stoppered bottle to prevent moisture changes and other contaminations and it was used for experiment.

Phytochemicaltsof *Docyniaindica*Decne.Peel and Pulp

Phytochemicals ofsampleswere investigated by using standard methods. (Harbone,1993)

Study on FT-IR Spectroscopy

The pure compound was isolated from 5 g of ethyl acetate extract ofeach sample by using thin-layer and column chromatographic methods. FT-IR spectra of pure compounds weremeasured in the range of 4000-450cm^{-1}in FT-IR spectroscopy.

Total Phenol Content by FCR Method

Preparation of standard gallic acid solutions

The stock solution of standard gallic acid (1 mg/mL) was prepared by dissolving 1mg of gallic acid in 1 mL of distilled water. This stock solution was twofold diluted serially with distilled water to get the standard gallic acid solutions with the concentration of 125, 62.5, 31.25, 15.625 and 7.8125 μg/mL.

Procedure forconstruction of gallic acid standard curve

Firstly, 1 mL of different concentration of Gallic acid solution (125, 62.5, 31.25, 15.625, 7.8125 μg /mL was mixed with 5 mL of diluted F-C reagent (FCR: H2O, 1: 10) and incubated for 15 min. To each tube, 4 mL of 1 M sodium carbonate was added and the tubes were kept at room temperature for 30 minutes and the UV absorbance of reaction mixture was measured at λ_{max} 765 nm using Ultraviolet Visible Spectrophotometer.

Investigation of total phenol content as gallic acid equivalent in sample

The total phenolic content (TPC) in each sample was estimated by Folin-Ciocalteu method. Each extract (1 mg) was mixed with 1mL of distilled water. To this, 5 mL of F-C reagent (1:10) was added and incubated for 15 minutes. To each tube, 4 mL of 1 M sodium carbonate solution was added and the tubes were kept at room temperature for 30 minutes and the UV absorbance of reaction mixture was measured at λ_{max} 765 nm using Ultraviolet Visible spectrophotometer.

Antimicrobial activity

The petroleum ether, ethyl acetate, ethanol, methanol and water extracts of peel and pulp of *Docyniaindica*Decnewere investigated for antimicrobial activity against the microbial strains *Bacillus pumilus, Bacillus subtilis, Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus*and*Candida albicans* using agar disc diffusion method at Development Centre for Pharmaceutical and Food Technology (DCPFT), Yangon.

Screening of Antioxidant Activity of Ethanol and Water Extracts of peel and pulp of *Docyniaindica*Decneby DPPH (1, 1-diphenyl-2-picryl hydrazyl)assay

Preparation of 60 M DPPH solution and test sample solution

DPPH (2.364 mg) was thoroughly dissolved in EtOH (100 mL). This solution was freshly prepared in the brown coloured reagent bottle and stored in the fridge for no longer than 24 hours.

Sample (2 mg) and 10 mL of ethanol were thoroughly mixed by shaker. The mixture solution was filtered and the stock solution was obtained. The sample solution (20, 10, 5, 2.5, 1.25 and 0.625 μg/mL concentration) was prepared from this stock solution by dilution with appropriate amount of ethanol.

The control solution was prepared by mixing 1.5 mL of 60 M DPPH solution and 1.5 mL of EtOH using shaker. The test sample solution was also prepared by mixing thoroughly 1.5 mL of 60 M DPPH solution and 1.5 mL of each sample solution. The mixture solutions were allowed to stand at room temperature for 30 minutes. Then, the absorbance of these solutions was measured at 517 nm by using UV-Visible spectrophotometer.

$$\%\,\text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

This formula is the calculation of percent inhibition of (IC_{50}) value. The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function.

Results and Discussion

Evaluation of Preliminary Phytochemicalsof peel and pulp of *Docyniaindica*Decne.
Theresults of preliminary phytochemicalsof samplesare listed in Table1.

Table1.Results of Preliminary Phytochemicals of peel and pulp of *Docyniaindica* Decne.

No:	Chemical Constituents	Test Reagent	Observation	Inference	
				Peel	Pulp
1	Alkaloids	Dragendroff'sreagent	orange ppt	+	+
2	Amino acids	Ninhydrin reagent	Purple color	+	+
3	Carbohydrates	10%α-Naphthol +conc:H_2SO_4	Red ring	+	+
4	Glycosides	10%Lead acetate	White ppt	+	+
5	Flavonoids	Hydrochloric acid+Mg	Pink color	+	+
6	Phenolic compounds	1%Ferric chloride	Deep green color	+	+
7	Reducing Sugar	Benedict's soloution	Deep yellow	+	+
8	Saponins	Distilled water	Frothing	+	+
9	Tannins	Ferrous sulphate	Pale balck	+	+
10	Starch	Iodine solution	Bluish-black ppt	+	+

(+) Presence,ppt= precipitate

Study on FT-IR spectrum

The FT-IR spectra of pure compounds of *Docyniaindica*Decne.peel andpulp are described in Figure2, which are identical with each sample.

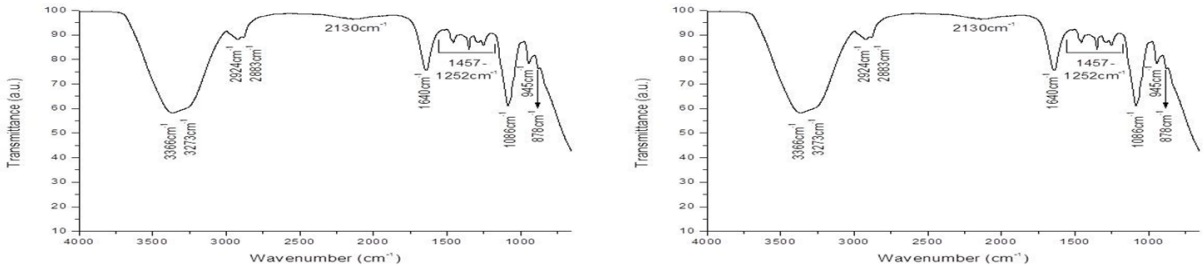

Figure 2.FT-IR spectra of pure compound of*Docyniaindica*Decne.peel and pulp

FT-IR spectra of pure compound isolated from the both samples indicated the presence of phenolic compounds which agreed with standard FT-IR spectrum of phenolic compound.

Total Phenol Content of Crude Extracts of *Docyniaindica*Decneby Folin-Ciocalteu Reagent (FCR).

Gallic acid standard curve,Figure 3 is required to evaluate the total phenol content of the selected sample. Because gallic acid is 3, 4, 5- trihydroxy benzoic acid and it is a kind of total phenol.

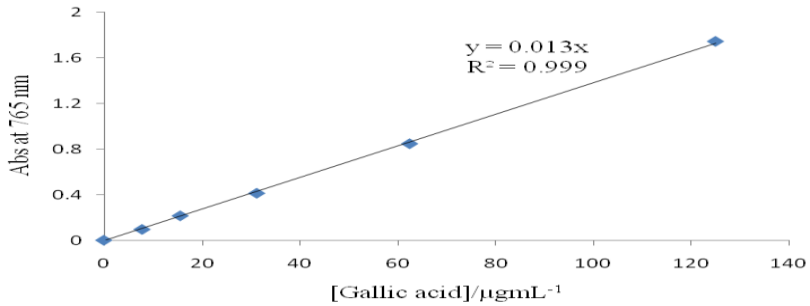

Figure 3. Gallic acidstandard graph

The results of total phenol contents for ethanol, and water extracts are described in Table2.

Table 2.Total Phenol Content of Ethanol, and WaterExtracts of *Docyniaindica*Decne.ByFolin-Ciocalteu Method

Sample	TPC (µ g GAE ± SD) / mg	
	EtOH	Water
Peel	34.15± 0.81	25.39± 2.81
Pulp	44.35± 0.51	38.71± 0.31

(Data expressed as (µg Gallic Acid Equivalents GAE, (mean SD) in 1 mg of sampleextract)

According to experimental data,ethanol extracts of selected both samples were observed to be more total phenol content than water extract of both samples.

Antimicrobial activity

The results of Antimicrobial activities of both samplesof the zone of inhibition are described in Table3a and 3b.

Table3a. The results of antimicrobial activities of *Docyniaindica*Decne Peel

Sample	Inhibition zone diameters of various solvent extracts against organisms						
	Solvents	I	II	III	IV	V	VI
Flower	Methanol	-	+	-	-	+	-
	Ethyl acetate	+++	+++	+++	+++	+++	+++
	Ethanol	+	+	++	-	+	+
	Aqueous	++	-	++	-	+	+
	Petroleumether	-	-	-	-	-	-

Table3b. The results of antimicrobial activities of *Docyniaindica*Decne Pulp

Sample	Inhibition zone diameters of various solvent extracts against organisms						
	Solvents	I	II	III	IV	V	VI
Flower	Methanol	-	+	-	-	+	-

	Ethyl acetate	+++	+++	+++	+++	+++	+++
	Ethanol	+	+	++	-	+	+
	Aqueous	++	-	++	-	+	+
	Petroleumether	-	-	-	-	-	-

Agar-well ~ 10 mm
10 mm ~ 14 mm (+) =low activity I = *Bacillussubtilis*
15 mm ~ 19 mm(++) = medium activityII = *Staphylococcus aureus*
20 mm above(+++)= high activity III = *Pseudomonas aeruginosa*
IV = *Bacilluspumilus*
V = *Candidaalbicans* and VI= *Escherichia coli*,
(–)= absent

According to these results, ethyl acetate extracts of both samples show high activity on all tested organisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans,*and *Escherichia coli*. Petroleum ether extracts samples show no activity on six selected organisms and methanol, ethanol and aqueous extracts exhibit low antimicrobial activity showed the presence of phenolic compound. Therefore, both samples contain bioactive compounds.

Antioxidant Activity of Ethanol, Methanol and Water Crude Extracts of *Docyniaindica*Decneby DPPH Radical Scavenging Assay

The results for antioxidant activity of samplesare shown in Table 4.

Table4.RadicalScavengingActivity (IC_{50}) of EtOH, and Water Extracts of *Docyniaindica*Decne.Peel and Pulp and Standard Ascorbic Acid

Extracts	**% RSA (mean ±SD) in different concentration (µg/mL)**						**IC_{50}** (µg/mL)
	0.625	**1.25**	**2.5**	**5**	**10**	**20**	
Water (peel)	22.02	28.92	33.07	36.88	44.78	54.95	15.14
	±0.76	±0.92	±0.51	±0.41	±0.53	±0.31	
Ethanol (peel)	36.19	55.89	66.45	75.82	87.12	91.99	0.86
	±1.01	± 0.36	± 0.57	± 0.62	± 0.51	± 0.30	
Ethanol (pulp)	19.98	29.52	41.3	57.76	71.62	76.81	3.82
	± 0.88	± 0.6	± 1.97	± 1.37	± 0.46	± 0.4	
Water (pulp)	22.20	30.52	43.53	57.82	73.32	79.21	1.59
	± 1.4	± 0.88	± 1.13	± 1.01	± 0.13	± 0.63	
Ascorbic acid	25.2	53.58	65.53	74.82	83.32	91.21	1.17
	± 1.4	± 0.88	± 1.13	± 0.59	± 0.78	± 0.48	

According to experimental result, the concentration of sample was increased, the % RSA was also increased.Antioxidant activity was found to be highest in waterextractwhich compaired withethanol extractand standard ascorbic acid in *Docyniaindica*Decnepulp. Antioxidant activity was found to be highest in ethanol extract which compaired with water extract and standard ascorbic acid in *Docyniaindica*Decnepeel. Therefore, *Docyniaindica* Decnepeel and pulpare suitable not only ediblebut also to use as medicine for human health.

Conclusion

This study showed the presence of active phytochemicals, antimicrobial and antioxidant activities of *Docyniaindica*Decnefruits are suitable to edible and can be used as medicine for healthbenefits.The present paper contributed important information for the further application of*Docyniaindica*Decne peel and pulp.

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