Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Chemistry

Elixir Appl. Chem. 127 (2019) 52629-52632

ISSN: 2229-712X

Analysis and Applications of Custard Apple

Ajitha Priya Neeraja Jammala, Ahlam 'Abdallah Salim Al-Shibli, Eman Hamad Zahran Al-Shuraiqi, Shahla Soud Abdullah Alrahbi and Zianab Mahmood Nasser Al Qassabi

Lecturer in Chemistry, Department of Applied Science, Higher College of Technology, Muscat Sultanate of Oman.

ARTICLE INFO

Article history:

Received: 24 December 2018;

Received in revised form:

03 February 2019;

Accepted: 13 February 2019;

Keywords

Custard Apple,

Pancreatic Cancer,

vitamin C,

DPPH.

ABSTRACT

Chemistry of natural product has contributed significantly towards the improvement of modern medicine. Custard apple (Annona squamosa) is a nutritional rich fruit and is largely valued for its taste. It is known to have some active ingredients which kills lung, colon, breast and pancreatic cancer cells. Therefore, consumption of this fruit is known to have aided in anti-cancer activities. The investigation is carried out to find the chemical composition, phytochemical properties and evaluation of crust, pulp and the seed of custard apple. The chemical characteristics such as pH, titratable acidity, total sugar and lipids are also verified. The results revealed that seed, pulp and crust contain adequate amount of vitamin C, citric acid, carbohydrate, protein etc. The phytochemical analysis revealed that custard apple contains tannins, flavonoids, cardiac glycosides and steroids. Standard GC analysis of seed oils contain certain types of essential oils which are mainly aiding in anti-inflammatory, anti-cancer and anti-microbial properties. The antioxidant activity is evaluated using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay. The presence of anthocyanins may also offer anti-cancer, anti-inflammatory and anti-viral benefits of custard apple.

INTRODUCTION

Humans over the ages searched, experimented and lived on nature's wonderful gifts like fruits and vegetables. They would consume them raw or as cooked food for their survival. (Quillin, 1999). All food contains more than just essential nutrients. Today a healthy diet rich in fruits and vegetables is very important, because it is a well-known fact that fruits contain vitamins, minerals, antioxidants, fibers, and small amounts of fat and calories and have a significant role in the cure of many diseases (Patil and Thorat, 2015). The highly complex nature of fruits results in abundance of desired and undesired reactions which are contributed by a variety of parameters. Among such is a custard apple which is a fruit native to tropical and subtropical areas. It has white custard like pulp surrounding small black seeds 3 to 5 inches in diameter with a bumpy green or red crust. The sweet flesh is eaten or used for milkshakes and ice creams.\

Figure 1. Custard apple fruit (Fruitinfo.,2004).

Crust Pulp Seed

Figure 2. Main compositions of custard apple fruits (Fruitinfo. 2004).

Physical and Chemical properties of custard apple fruits

Custard apple fruit tree is a small tree which can grows wild in different places across the globe. The fruit is oval-shaped and almost a bit heart-shaped.

It will be grown up to 10 cm wide and 10 to 20 cm long. It can weigh anywhere from 140 -510 grams and some custard apples have weighed 5 pounds (2 1/4 kilograms).The fruit has a rough leathery skin, which overlaps on itself in scales and ripens to a pale green or creamy yellow. The taste of pulp is somewhere between pineapple and banana papaya, while some others compare the taste to match sweet pear. The flesh inside is segmented and in each segment there is a large, single, hard shiny black seed about the size of a bean which is removed while eating. The fruit is picked when it is firm and let to ripen at room temperature until it yields to gentle pressure.. It consists of water soluble vitamins and fat soluble vitamins. The custard apples are a good source of calcium, iron and phosphorus and contain many minerals and nutrients that are beneficial for a healthy life. It has been tested for its nutritional values and known to have nutrition for every 100 g of the fruit as calories (80-101), protein 68g, fat 0.5g, carbohydrate 20g, fiber 0.9 g, Iron 0.42 mg., carotene 0.007 mg ,thiamine 0.075 mg, phosphorous14.7mg, calcium 17.6 mg, riboflavin 0.086 mg, nicotinic acid 0.528mg and vitamin C 15 mg.(Gyanunlimited,2013).

Tele:

E-mail address: ajjitha@gmail.com

Medicinal uses

Eating custard apple will help protect human body from many diseases and disorders. It is good for bone, skin, and heart, and maintains a steady healthy blood pressure. The paste of the flesh of custard apple is used to treat boils and ulcers. The dried crushed parts inpowdered form of are good in curing diarrhea. In addition to the fruit, other parts of the custard apple tree hold several medicinal properties. The bark of the custard apple tree is usedby herbal medicine manufacturers in the treatment of toothache (Nair, 2014). The fruitis one of the best source of compounds called (acetogenins) which are long chain fattyacids that have anti-cancer properties. Furthermore, the antioxidants in custard apple destroythe harmful free radicals in humans and prevent cell damage. Presence of anthocyanins, a powerful antioxidant makes this fruit even more beneficial.

Validation

The range of the proposed procedure is the interval between the upper and the lower value in the sample for which it has been demonstrated that the methods have suitable levels of precision and accuracy. The statistical analysis for evaluation of the method performance includes the parameters such as mean, standard deviation & variance, T-test, F-test.

Aim and Objective

The purpose of this project is to find the chemical compositions such as reducing sugars carbohydrates, proteins, fats as well as vitamin C content, acidity, amount of sodium. Antioxidant properties are verified by DPPH assay. GC-MS assay also gave results to confirm the amount of fatty acids present in these fruits. The presence of anthocyanins, a powerful antioxidant, and total anthocyanins are determined using AOAC method.

MATERIALS AND METHODS

To measure the moisture content, water activity and shelf life of custard apple, The dried crucibles labeled and weighed accurately. 10 g of ground sample was put in the crucible and weighed accurately. The sample was placed in oven at 105 ^{0}C for 8 hrs then cooled in desiccator. The samples were weighed. Repeated drying until stable weight was obtained. The percentage of moisture was calculated.

Quantitative analysis of sugars

The concentration of reducing sugars in the crust, pulp and seed of Custard apple samples were found using range of color standards. 5.0 cm^3 of Benedict's reagent is taken into a test tube and 3 cm^3 of the solution was added to be tested. Kept in a boiling water bath for 8 minutes, cooled then compared the color produced with the color of the standards.

Qualitative analysis of carbohydrates and proteins

The presence of Carbohydrates in the fruit crust, pulp and seed was verified by conventional methods like Molisch's test, Fehling's test and Iodine test. The proteins were identified by using Millon reagent.

Quantitative measurement of invisible fat from food

2.5 g of each dried sample (crust, pulp and seed) was weighted, crushed and broken into dime- size pieces between two sheets of foil with a mortar. The samples were transferred into the beakers which were labeled and weighed. 10 ml of acetone was added to the crushed samples, swirled for 1 minute in a hood (ventilated area). Carefully the acetone was decanted into a petri dish making sure the sample remained in the beaker. The acetone in the petri dish was allowed to dry overnight in a hood to visualize the lipid that was extracted.

Titratable Acidity and pH of custard apple

A standardized solution of NaOH was used to titrate the organic acids in the fruits using phenolphthalein indicator and determined the titratable acidity. 5 ml of custard apple sample solution (crust, pulp and seed) were pipetted into a 250 ml beakers. 25 ml of distilled water was added to each beaker and added 3 drops of phenolphthalein solution. Then the sample was titrated against 0.1N NaOH solution and the pH was measured before and after the end-point was determined and then the acidity was calculated.

Determine the amount of Vitamin C in custard apple

1- A standard solution of ascorbic acid was prepare only at time of use:

50 mg ascorbic acid was weighed accurately and transfer to a 50 ml volumetric flask. It is diluted immediately before use with the metaphosphoric acid acetic acid solution.

2-Metaphosphoric acid acetic acid solution:

To a 250ml beaker 100 ml distilled water was added then 20ml acetic acid. To this 7.5g metaphosphoric acid is added and stirred to dissolve. It was diluted to 250 ml with distilled water, closed the bottled with a stopper and refrigerated until use.

3-Indophenol solution dye:

To 50ml deionized distilled water in a 150 ml beaker, added 42 mg sodium bicarbonate and 50mg 2, 6-dichloroindophenol sodium salt, stirred to dissolve. Diluted the mixture to 200 ml with distilled water. Filtered through fluted filter paper into an amber bottle and refrigerated until use.

4-Standardization of Dye and analysis of the samples:

5 ml metaphoshoric acid acetic acid was pipetted solution into each of three 50ml Erlenmeyer flasks. Then 2 ml ascorbic acid standard solution is added to each flask. It is titrated against indophenol dye solution until a light but distinct rose pink color persists. The burette readings are noted. Repeated steps 3-5 for the other two standard /samples. Recorded the initial and final burette reading and calculated the volume of dye used for each sample. Also titrated the blank in the same way and calculated the volume of dye used.

Phytochemical analysis of seed and pulp of custard apple extract

1. Test for flavonoids

Shinoda test: To the test solution a few magnesium turnings, a few drops of concentrated hydrochloric acid were added. Pink color crimson red/occasionally green to blue color appeared after few minutes.

2. Test for tannins

Ferric chloride test: The extract was treated with ferric chloride solution blue color appeared if hydrolysable tannins are present and green color if condensed tannins are present.

3. Test for cardiac glycosides

Keller- KillaniTest: 5ml of sample was dissolved in 5ml of water and 2ml glacial acetic acid with 3ml ferric chloride solution were added. Further 6 drops of concentrated sulphuric acid was added. A brown color ring appeared at the junction of two liquids.

4. Test for steroids

Salkowskitest 5ml of sample solution was mixed with few ml of chloroform and shaken with concentrated sulphuric acid. After standing some time it gave red color.

Oil Extraction

50 g of grounded seed was weighed and transferred to a 30 mm × 200 mm cellulose thimble. It was placed in a 250 ml soxhlet apparatus fitted with condenser. It was placed on 500 ml distillation flask containing 250 ml of solvent (n-

hexane). Custard apple seed oil was extracted with n-hexane for six hours. Hexane was then removed by used heated rotary evaporator. This oil extracted is used for both GC-MS assay and DPPH assay.

GC-MS analysis

0.5 ml from sample was taken into GC vials and added 0.5 ml of hexane, analyzed for the presence of various compounds.

DPPH Radical Scavenging Activity

5mg of DPPH solid is dissolved in methanol and made up to the mark in a 250 ml volumetric flask. 5 test tubes were taken in order and added different amount of samples (90, 100,120,140 µl) and 5ml of DPPH solution is added to each test tube, kept at room temperature for 10 min . Observed the change in color then after that absorbance was measured at 520nm using UV spectrophotometer. Then, the percentage inhibition was calculated by the following formula,

$$I\ (\%) = \frac{A^\circ - A1}{A^\circ} \times 100$$

I(%)is precentage inhibtion

A° is the absorbance of the control

A1 is the absorbance of custard apple oil

Figure 3. Oil extracted.

Figure 4. DPPH solution

Total Monomeric Anthocyanin Content (AOAC Official Method) 2005.02 is used.

Anthocyanin is extracted and total monomeric content is determined by the method described in AOAC. Then the liquids separated completely and drained the lower aqueous layer into 15 ml conical graduated centrifuge tube. To 0.2-0.5ml portions, H_2O is added to separator and centrifuged each time and removed lower layers until a total of 2.5 ml was collected. Then, mixed the solution and used 0.5ml for anthocyanin test. 0.1ml of anthocyanin solution was pipetted into small flask and added 10ml eluting solvent and mixed and the absorbance was measured at 545 nm. The percent by weight of anthocyanins was calculated using the formula,

$$\%w/w = \frac{A}{\in L} MW \frac{v}{w} 100$$

A= Absorbance

$$A = (A_{510nm} - A_{700nm})_{pH=1} - (A_{510nm} - A_{700nm})_{pH=4.5}$$

RESULTS AND DISCUSSION

Table 1. Data for some properties.

Property	moisture content	reducing sugars	lipid content	Citric acid	Ascorbic acid/ Vitamin C mg/ml
Crust	60%	1-2%	4.60%	9.15%	6.84
Pulp	74%	2-5%	1.72%	10.1%	9.83
Seed	71%	0.5-1%	11.36%	3.60%	14.9

Results in Table 1 reveal that pulp contains highest amount of moisture, reducing sugars, citric acid and ascorbic acid content while the lipid content is very low in the pulp. It can be seen that the seed has the highest concentration of lipid content as well as Vitamin C.

The large amount of water in custard apple gives the fruit less shelf life due to the growth of microorganism after 2-3 hours at room temperature.

Identification of Carbohydrates and proteins

Iodine test for the presence of starch was positive whereas the Molisch's and Fehling tests gave negative results for all the three samples. It was also observed brick-red color precipitate with Millon's reagent indicated the presence of protein in the pulp and crust.

Phytochemical analysis of seed and pulp sample

Table 2. Phytochemical Tests.

Types of test	Seed samples	Pulp samples
Test for flavonoids: Shinods test	Negative test	Positive test
Test for tannins Ferric Chloride test	Negative test	Positive test
Test for Cardiac glycosides Keller- Killani test	Negative test	Positive test
Test for steroids Salkowski test	Negative test	Positive test

The results of phytochemical screening conducted are shown in the Table: 2, which gave positive results in pulp samples and negative results in the seed samples. This indicates the presence of flavonoids, tannins, cardiac glycosides and steroids in the pulp. The flavonoids are synthesized in plants and act as anti-microbial, anti-inflammatory, anti-cancer, antioxidant and tumor inhibitory. (Rattana et. al., 2010)The antimicrobial activities of tannins are well fixed. The growth of many fungi, yeasts, bacteria and viruses are inhibited by tannins. Cardiac glycosides are one of several classes of drugs used to treat the heart and related conditions. Steroid is a type of organic compounds that contains specific arrangements of four rings that are joined to each other.

Therefore, the presences of these medicinally bioactive substances in the pulp make the fruit even more beneficial for human health.

Oil Extraction

The sample of seed oil from extraction was collected and kept it in a small container with aluminum foil covered to prevent the light enter the sample and disturb their components.

GC MS analysis

Table 3. Retention time for different components.

Retention time	Name of compound
4.901	Caryophyllene
5.540	Alpha.-Humulene
6.064	Germacrene D
6.374	Bicyclogermacrene

GC MS analysis revealed that the major components present in the sample are Caryophyllene, Alpha.-Humulene**,** Germacrene D and bicyclogermacrene respectively are all non-oxygenated sesquiterpenes.

Caropyllene is a natural bicyclic sesquiterpene, a constituent of many essential oils. It is anti-inflammatory in nature. Alpha-humelene has been known to be an anti-cancer agent. In 2003, it was discovered that humulene helps produce reactive oxygen species, which are chemicals that help destroy cancer cells through apoptosis, a process by which cells kill themselves in a pre-programmed death ritual. A 2007 study published in the Journal of Pharmacy and Pharmacology discovered that the terpenes beta caryophyllene, BCP and humulene work together to kill cancer cells. In simple terms, BCP amplifies the anti-cancer effects of humulene. Further, the European Journal of Pharmacology illustrated the anti-inflammatory properties of humulene. In 2009, The British Journal of Pharmacology again examined the anti-inflammatory characteristics of humulene and concluded that this terpene to be effective against inflammation when consumed either orally or by aerosol. The most significant remedial properties of Germacrene D are its antibacterial properties. Many studies indicated that when oils contain Germacrene D and caryophyllene, then they mainly are responsible for the cytotoxic effects. Further studies are required to emphasize the importance of non-oxygenated sesquiterpenes and their interactions.

DPPH Radical Scavenging Activity

The ability of the essential oil to scavenge DPPH radical was calculated as DPPH scavenging effect or % inhibition by the following equation: % inhibition = {(Abs control – Abs sample)}/(Abs control) × 100

Table 4. Inhibition Percentage

Volume of extracted oil (microliter)	Absorbance	*I*(%)
90 µl	0.196	**17.99**
100 µl	0.185	**22.59**
120 µl	0.12	**49.79**
140 µl	0.3	**87.4**

The data in Table 4 show that the inhibition percentage is increasing. The antioxidant potential by DPPH radical scavenging method showed that the custard apple seed oil is having a better antioxidant activity.

Total Monomeric Anthocyanin

Total anthocyanin was found to be 6.5 mg/l for the pulp. This indicated that anthocyanin is present in custard apple fruit and are responsible for antioxidant properties.

Conclusion

Certain properties of custard apple were verified through both qualitative and qualitative analysis. The results confirmed that the fruit contains a favorable amount of moisture, reducing sugars, lipids and vitamin C and other useful acids. Further, phytochemical properties like flavonoids, tannins, cardiac glycosides and steroids were found to be present. Its oxidizing capacity was confirmed by DPPH assay. GC MS assay and the presence of total anthocyanins in fruit confirmed that it has substances which aid in anti-cancer, anti-inflammatory, antioxidant activities. Further studies are required to check and compare the different anticancer drugs as against custard apple's ability as a natural source to be used in the treatment of cancer.

References

1.http://www.fruitscapesllc.com/fruitCUSTARDAPPLE.html
2.Fruitinfo.(2004).*Custard Apple* .Retrieved November 15, 2017
3.http://www.fruitsinfo.com/SugarApple-fruit.php
4.Belitz, H.-D., Grosch, Werner, Schieberle, Peter, (2009), Food Chemistry, copyright 1999 Springer-Verlag Berlin Heidelberg.
5.Reena Nair and Vijay Agrawal, 2017. A Review on the Nutritional Quality and Medicinal Value of Custard Apple-An Under Utilised Crop of Madhya Pradesh, India, International Journal of Current Microbiology and Applied Sciences. 6:1126-1132.
6.www.gyanunlimited.com/health/custard-apple-health-benefits-and-nutritional-facts
7.Chandran Prince Jebadass Isaac &A. Sivakumar, 2013. Removal of lead and cadmium ions from water using Annona squamosa shell: kinetic and equilibrium studies, 7700-7709
8.Himangshu Sekhar Maji Maji,S.(2016). Analytical and Phytochemical Exploration of the Seeds of Annona Squamosa, Journal of Analytical & Pharmaceutical Research, 3:4.
9.http://www.valuefood.info/1665/health-benefits-of custard-apple/
10.Patil and Thorat .(2015). Standardization ofPretreatments for Liquefaction ofCustard Apple for Spray Drying. A Thesis submitted to the Mahatma Phule Krishi Vidya Peeth, Rahuri-413 722, Dist. Ahmednagar, Maharashtra, India.
11.Quillin,P.(2005). *Beating Cancer with Nutrition*. Nutritin time press, United State of America.
12.Umesh and Vishwas.(2014). Phenolic Composition And Antioxidant Capacity Of Wine Prepared From Custard Apple (Annona Squamosa L.) Fruits,*Journal Of Food Processing And Preservation,* 39:2:175-182.
13.Germacrene-D, a characteristic component of the Essential Oils from the Leaves of Juniperusoxycedrus ssp. Macrocarpa (S. et Sm.) BalI Growing in El Kala, (2016), Journal of Natural Products And Plant Resources, Algeria
14.Marcos José SalvadorI,*; João Ernesto de CarvalhoII; Alberto Wisniewski-JrIII; Candida A. L. KassuyaIV; Élide P. SantosV; Dilamara RivaVI; Maria Élida Alves Stefanello VI,(2011)Chemical composition and cytotoxic activity of the essential oil from the leaves of *Casearialasiophylla*, Revista Brasileira de Farmacognosia, Vol.21 no.5.
15.Essential oil constituents, anticancer and antimicrobial activity of Ficusmucoso and Casuarinaequisetifolia leaves
16.The Plant SesquiterpeneGermacrene D Specifically Activates a Major Type of Antennal Receptor Neuron of the Tobacco Budworm Moth Heliothisvirescens
17.Rattana, Surapong & Phadungkit, Methin & Cushnie, Benjamart. (2010). Phytochemical Screening, Flavonoid Content and Antioxidant Activity of TiliacoraTriandra Leaf Extracts.