Antioxidant activity and phytochemical compounds of snake fruit (Salacca zalacca)

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Abstract. Snake fruit (Salacca zalacca) is a palm tree species, which is found in Malaysia and Indonesia. This study was conducted to investigate and compare the composition, total phenolic, flavonoid, tanins and monoterpenoids contents in the core and shell fruits. Concentration values of extracts were obtained from standard curves obtained. Antioxidant activity was determined using DPPH method. For all methods it was used the UV-VIS Specord M40, using different wavelength. The infrared spectral analysis was carried out to caracterized the type of functional group existent in snake fruit parts (shell and core).

1. Introduction

Scientifically studies confirmed the fact that consumption of fruits and vegetables reduce incidence of different types of diseases, like cancer or cardiovascular disease [1, 2]. They contain a number of types of minerals and phytochemicals (phenols and flavonoids) that provide important health benefits [3-6]. Nowadays a variety of tropical fruits are found in Europe. The high nutritional value of the subtropical and tropical fruits led to significant increase in their consumption, but a lot of people are not familiar with the nutritional values of these fruits [7-9].

Snake fruit (Sallaca zallaca) is a specie of a palm tree, from family Arecaceae, is a good source of natural antioxidants, which containing many different radical scavenger [9, 10]. This fruit it is also called “The Future of Our Health” and “The Superheroes of Functionality”. The skin fruit is brown and looks like a snake skin. The fruit has an astringent taste [11,12].

Leontowicz et al [3] discovered that snake fruit has the ability to prevent atherosclerosis in vivo and it has a significantly displayed higher antioxidant value than mangosteen.
The snake fruit resembling an egg like in shape and the skin of the mature fruit is brown. The fruit contain a kernel covered with white flesh. The aroma it is between pineapple, pear and banana. The weight is up to 70 g at the last maturation stage. Most of this fruit is freshly consumed and some are processed into fruit juice, canned fruit or jam [10-13]. This research presented the snake fruit characteristics and benefits for human body. The results indicate that snake fruit contain certain amounts of polyphenols, flavonoids, tannins and monoterpenoids, proving them to be perfect sources of antioxidants.

2. Materials and methods

2.1. Materials
Snake fruit was added from Bali Island (Indonesia). Standard substances used for curve calibration of methods are linalool (Merck), vanillin (Scharlau), gallic acid (Merck), catechin () and 2,2-diphenyl-1-picryl-hydrazyl-hydrate stable free radical (DPPH from Merck); methanol by Scharlau. For determinations of methods it was used also aluminium chloride (AlCl₃ from Sigma-Aldrich), NaNO₂, NaOH, H₂SO₄ (Merck), sodium carbonate (Na₂CO₃ from Merck), HCl and Folin–Ciocalteu reagent (Merck).

2.2. Preparation of plant sample
The Snake fruit (Sallacca zallacca) was peeled and cut into small pieces. The fruit (figure 1, a) core and b) shell) was weighed and exhaustively extracted in hidroalcoolic mixture (CH₃OH : H₂O dist.; 9:1), at room temperature for 24 hours. The macerate was kept in a brown volumetric dark flask in order to avoid degradation.

2.2. Characterization Methods

2.2.1. UV-VIS Spectroscopy. The absorption spectra of the samples were recorded on a double beam M 400 Carl Zeiss Jena UV-VIS spectrophotometer from 400 to 800 nm, at the resolution of 1 nm, with 1 nm slit width and 0.3 nm/s scan rate.
2.2.2. **Fourier transform infrared spectroscopy.** The functional groups of the snake fruit (shell and core) were determined using a Vertex 80 FTIR spectrometer with the Hyperion 3000 IR microscope.

2.2.3. **Phytochemical Analyses.** The phytochemical quantification procedures were used for the determination of total tannins, total flavonoids, total pholyphenols and total monoterpenoids in the snake fruit extracts. The assays are presented in table 1.

| No. | Assay              | Reagents                                      | Conditions                                      | Monitoring system and standard curve | Ref.     |
|-----|--------------------|-----------------------------------------------|-------------------------------------------------|--------------------------------------|----------|
| 1   | Total Tanins       | 0.5 mL extract + 3 mL 4% vanillin-MeOH + 1.5 mL HCl | 15 minutes of incubation at room temperature | Absorbance at 500 nm; Catechin curve calibration standard | [14,15]  |
| 2   | Total flavonoids   | 1 mL extract + 4 mL distilled water + 0.3 mL NaNO2 (5%); After 5 minutes, 0.3 mL AlCl3 (10%); After 5 minutes, 2 mL 1M NaOH + 2.4 mL distilled water | 30 minutes of incubation at room temperature | Absorbance at 510 nm; Catechin curve calibration standard | [15-17]  |
| 3   | Total polyphenols  | 1 mL diluted extract + 5 mL Folin-Ciocalteau reagent. After 8 minutes, 4 mL Na2CO3 | 60 min of incubation at room temperature | Absorbance at 765 nm; Gallic acid curve calibration standard | [16-18]  |
| 4   | Total monoterpenoids | - 2 mL extract + 1 mL 2% vanillin-H2SO4 | - heated at 60 °C/20 min, - cooled at 25 °C/5 min | Absorbance at 608 nm; Linalool curve calibration standard | [19]     |

2.2.4. **Antioxidant activity determination.** The principle of this method consists in reducing in the presence of an antioxidant molecule, giving rise to coloured methanol solutions. The utilization of DPPH method delivers an easy and rapid mode to estimate antioxidant activity against free radicals [20, 21]. DPPH solution is a methanolic solution prepared in lab (0.02 mg DPPH/mL MeOH), with violet colour. From each sample it was used 0.5 ml extract sample and it was mixed with 1 mL of DPPH solution. After it were agitated and it were left in the dark for an hour, the mixtures were tested by reading the absorbance $\lambda$=517 nm on a UV-VIS Specord M 400 spectrophotometer. As a blank, it was used a mixed solution prepared from 0.5 mL of MeOH with 1 mL of 0.02 mg/mL DPPH solution, which it was measured at the same wavelength. The antioxidant activity (AA%) percentage was calculated using the formula [20, 21]:

$$AA\% = \left(\frac{A_{Control} - A_{Sample}}{A_{Control}}\right) \times 100$$

where: $A_{Control}$ = absorbance of a DPPH solution without sample; and $A_{Sample}$ = absorbance of the sample mixed with 0.02 mg/mL DPPH solution.
3.1. UV-VIS results
UV-visible spectroscopy demonstrated to be very useful technique for the analysis of extracts. The UV-Vis analysis (figure 3) shows the UV-VIS spectra obtained for the snake fruit core and shell extracts, identified the maxima wavelengths specific flavonoids and other phenolic compounds at 280-300 nm and 300-350 nm. The peaks of hydroalcoholic extracts characterized by absorptions in the UV region 250-450 nm, corresponding to phenolic acids and their derivatives (flavones, flavonols, phenylpropenes, quinones). At 280 nm it is identified the specific wavelength for phenolics acids and between 300-350 are found flavonoids, quinines, coumarins [22-24].

![UV-VIS spectra of Salacca zalacca extract](image)

Figure 3. The UV–VIS spectra of Snake fruit extract.

3.2. FTIR results
The infrared spectral analysis was carried out to characterized the type of functional group existent in snake fruit parts (shell and core). FTIR spectra of components from snake fruit is used to observe the type of functional groups contained in this type of fruit. The spectrum was measured at a wavenumber range between 200 - 4000 cm$^{-1}$ and the results are expressed in figure 4 (a) and b)).

FTIR spectrum from fig.5 a), presented shell snake fruit extract from outside (ext) and inside (int). Are observed strong bands at 3225 cm$^{-1}$ attributed to hydroxyl groups and the band is more pronounced for exterior part of shell snake fruit. The band from 2950 cm$^{-1}$ is characteristic to groups CH. The bands C = C and C = O are found at 1590-1458 cm$^{-1}$ region. The aromatic group of the amide of type I and II are observed in the region between 1390 and 1320 cm$^{-1}$. The CO groups, esters, hidroxiflavones, catechins and amides of type III are visible at 1220-1150 cm$^{-1}$ regions, especially at exterior part of shell snake fruit, and the aliphatic amine functional groups are predominant in 1000-700 cm$^{-1}$ area. The shell snake extract expose weak IR bands between 1140-1075 cm$^{-1}$ specific for C\N stretching vibrations of aliphatic amines or C/O stretching vibrations of alcohols or phenols, which are found due to different types of phytoingredients present in the fruit extract (polyphenols, polysaccharides and proteins) [25-26].

Also, FTIR results for core snake fruit (fig. 4 b) presented carboxyl groups -C=O at 1647 cm$^{-1}$ band and OH absorption emerging appeared at 3465 cm$^{-1}$. Peak at 1321 cm$^{-1}$ is specific for -C-O of the alcohol. -CH group was observed at 2875 cm$^{-1}$. Carboxyl groups shows uptake -C = O appeared at 1500 cm$^{-1}$ and OH absorption emerging as a broad band at 3200 cm$^{-1}$. Peak at 1200 cm$^{-1}$ was the peak -C-O of the alcohol and peak at 3200 cm$^{-1}$ it is attributed to –CH group. The core snake fruit extract FTIR spectrum show a weak band at 1716 cm$^{-1}$ specific to amide I, which result from stretch vibration of carbonyl C-O from proteins, and a strong band at 1350 cm$^{-1}$ specific to C/O group of polyols: catechins or hydroxyflavones.

The short two bands observed in both parts (shell and core) of snake fruit extract, at approximate 2870 cm$^{-1}$ and 2980 cm$^{-1}$ are characteristic for \C\H asymmetric and symmetric stretching vibrations of saturated C (sp$^3$) [25-26].
3.3. Antioxidant activity results
In table 2, are observed the results of antioxidant activity determination. Fruit represents a higher percentage of antioxidant activity against shell. Antioxidant activity of samples extracted from snake fruit, shows high values, which demonstrates a high content of vitamins, beneficial for body.

| Sample          | AA%  |
|-----------------|------|
| Snake fruit - core | 82.675 |
| Snake fruit - shell | 73.135 |

3.4. Results of total flavonoids content
For preparation of the calibration curve, it was used Catechin as standard. It was prepared standard solutions of different concentrations: 20, 40, 60, 80 and 100 mg / g. The total flavonoids content (TFC) of the extracts was determined from the regression equation for the calibration curve \( y = 0.0003x +0.0054; R^2 = 0.9988 \).

The results of total flavonoid content are presented in table 3. It was observed a good flavonoid content in the both snake fruit samples (core and shell).
Table 3. Total flavonoids content in snake fruit

| Sample          | T.F.C. mg/g Catechin |
|-----------------|----------------------|
| Snake fruit - core | 129.8 ± 0.003        |
| Snake fruit - shell | 124.9 ± 0.004        |

3.5. Results of total tannins content

The amount of total condensed tannins is expressed as mg catechin/g DW. All samples were analyzed in triplicate and the results are presented in table 4. The total tannins content (TTC) of the extracts was determined from the regression equation for the calibration curve (y = 0.0003x +0.0054; R² = 0.9988).

Table 4. Total tannins content in snake fruit

| Sample          | T.T.C. mg/g Catechin |
|-----------------|----------------------|
| Snake fruit - core | 109.1 ± 0.0003        |
| Snake fruit - shell | 63 ± 0.0005          |

3.6. Results of total polyphenols content

For preparation of the calibration curve, it was used Gallic acid as standard. It was prepared standard solutions of different concentrations: 10, 20, 30, 40 and 50 mg / g.

The total polyphenols content (TPC) of the extracts was determined from the regression equation for the calibration curve (y = 0.0118x +0.0173; R² = 0.9944).

The total phenolics content presented in table 5 and calculated from the calibration curve, were 1067.79 gallic acid equivalents/g and 946.61 gallic acid equivalents/g. Research articles detailed the
fact that phenolic compounds from fruits have redox properties, which permit them to play as antioxidants [26].

| Table 5. Total phenolics content in snake fruit |
|-----------------------------------------------|
| Sample                        | T.P.C. mg /g Gallic acid |
| Snake fruit - core            | 1067.796 ± 0.0002        |
| Snake fruit - shell           | 946.610 ± 0.042          |

3.7. Results of total monoterpenoids content

For preparation of the calibration curve, it was used Linalool as standard. The total terpenoids content (TMtC) of the extracts was determined from the regression equation for the calibration curve ($y = 0.0139x + 0.3939; R^2 = 0.9999$).

![Linalool calibration curve](image)

Figure 7. Linalool calibration curve.

The results of total terpenoids content are detailed in table 6. The highest value was obtained for snake fruit core (223.89 mg/g), which is correct because in fruit are major terpenoids content, not in the core of this fruit.

| Table 6. Total monoterpenoids content in snake fruit |
|-----------------------------------------------|
| Sample                        | T.Mt.C. mg /g Linalool |
| Snake fruit - core            | 223,892 ± 0.06        |
| Snake fruit - shell           | 39,395 ± 0.0002       |

4. Conclusions

In the present study, it was determined total flavonoids, polyphenols, monoterpenoids, tannins and antioxidant activity of a tropical fruit, snake fruit, brought from Indonesia. Also, the fruit compounds were characterized using different types of analytical methods (FTIR, UV-VIS). The existence of phenolic compounds in the snake fruit was confirmed by the Folin-Ciocalteu method. ATR-FTIR results demonstrated the major amount of carbohydrates, aminoacids, proteins and of the phytoingredients and hydroxyl functional groups (polyphenols).

The antioxidant capacity was measured by the free radical scavenging methods DPPH. The methanolic solutions of the snake extract showed high antioxidant capacity (AA core = 82.67% and AA shell = 73.13 %). All results of phytochemical analyses were made in triplicate and calculated using results of calibration curves, with very good regression indices. In conclusion, the phytochemical estimation of the components of snake fruit, the results in this study indicate that snake fruit contain certain amounts of polyphenols, flavonoids, tannins and monoterpenoids, proving them to be perfect sources of antioxidants.
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References
[1] S. Southon, 2000 Food Res. Int., 33, pp. 211–217.
[2] P. Saiko, A. Szakmary, W. Jaeger and T. Szekeres, 2007 Mutat. Res., 658, pp. 68-94.
[3] M. Leontowicz, H. Leontowicz, J. Drzewiecki, Z. Jastrzebski, R. Haruenkit, S. Pooavarodom, Y. S. Park, S. T. Jung, S. G. Kang, S. Trakhtenberg and S. Gorinstein, 2007 Food Chem., 102 pp. 192–200.
[4] M. Oktay, I. Gülçin and O. Küfrevioglu, 2003 Lebensm.Wissen Technol., 36, pp.263-271.
[5] M. Karsheva, E. Kirova, S. Alexandrova and S. Georgieva, 2013 J. Chem. Tech. Metall., 48, pp. 475-478
[6] A. E. Hegazy and M. I. Ibrahim, 2012 World Appl. Sci. J., 18, pp. 684-688.
[7] A. Duda-Chodak and T. Tarko, 2007 Acta Sci. Pol. Technol. Aliment., 6, pp. 29-36.
[8] R. Gyawali and K. Su Kim. 2014 J. Appl. Anim. Res., 4, pp. 85-95.
[9] S. Gorinstein, R. Haruenkit, S. Pooavarodom, Y. S. Park, S. Vearasilp, M. Suhaj, K. S. Ham, B. G. Heo, J. Y. Cho and H. Gi Jang, 2009 Food Chem.Toxicol, 47, pp. 1884–1891.
[10] L. H. A. Priyatno, E. Y. Suka ndar, S. Ibrahim and I. K. Adnyana, 2007 J. App. Sci., 7, pp. 3127-3130.
[11] G. F. Deng, C. Shen, X. R. Xu, R. D. Kuang, Y. J. Guo, L. S. Zeng, L. L. Gao, X. Lin, J. F. Xie, E. Q. Xia, S. Li, S. Wu, F. Chen, W. H. Ling and H. B. Li, 2012 Int. J. Mol. Sci., 13, pp. 8308-8323.
[12] E. Munaf, F. Hayuni, R. Zein and H. Suyani, 2014 Res. J. Pharma. Biol. Chem. Sci., 5, pp. 1535-1543.
[13] T. Budiyanti, S. Hadiati, R. Prihatini and Sobir, 2015 Int. J. Adv. Sci. Engin. Info. Technol., 5, pp. 4.
[14] A. Rebaya, S. I. Belghith, B. Baghdikian, V. Mahiou Leddet, F. Mabrouki, E. Olivier, J. K. Cherif and M. T. Ayadi, 2014 J Appl Pharm Sci, 5, pp. 052-057.
[15] F. Medini, H. Fellah, R. Ksouri and C. Abdelly, 2014 J. Taibah. Univ. Sci., 8, pp. 216–224.
[16] S. A. Baba and S. A. Malik, 2015 J. Taibah. Univ. Sci., 9, pp. 449–454.
[17] S. Kaur and P. Mondal, 2014 J. Microbiol. Experiment, 1, pp. 6.
[18] International Standard ISO 14502-1, Determination of substances characteristic of green and black tea — Part 1: Content of total polyphenols in tea — Colorimetric method using Folin-Ciocalteu reagent, 2005-03-01.
[19] C. Indumathi, G. Durgadevi, S. Nithyavani and P. K. Gayathri, 2014 Int J ChemTech Res, 6, pp. 4264-4267.
[20] I. R. Bunghez, O. Dumitrescu, R. Somoghi, I. Ionita and R. M. Ion, 2015 Rev. Chim., 66, pp. 112-115.
[21] I. R. Bunghez, R. C. Fierascu, O. Dumitrescu, I. Fierascu and R. M. Ion, 2015 Rev. Roum. Chim., 60, pp. 515-519.
[22] H. Zhu, Y. Wang, Y. Liu, T. Xia Yand Tang. 2010 Food Anal. Method., 3, pp. 90–97.
[23] D. B. Rodriguez-Amaya, 1993 Chemical, biological, physical and nutritional aspects (Amsterdam, Elsevier) pp. 547-589.
[24] F. Bunghez, C. Socaciu, F. Zăgrean, R. M. Pop, F. Ranga and F. Romanciuc, 2013 Bull UASVM Food Sci. Technol., 70, pp. 16-24.
[25] M. E. Barbinta-Patrascu, I. R. Bunghez, S. M. Iordache, N. Badea, R. C. Fierascu and R. M. Ion, 2013 J. Nanosci. Nanotechnol., 13, pp. 2051-2060.
[26] I. R. Bunghez, M. E. Barbinta-Patrascu, N. Badea, S. M. Doneca, A. Popescu and R. M. Ion, 2012 J. Optoelectron. Adv. Mat., 14, pp. 1016-1022.