Antioxidant capacity of pineapple (*Ananas comosus* [L.] Merr.) extracts and juice

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Polyphenols are secondary plant metabolites with proven antioxidant activity, with an important role in prevention of many diseases. Pineapple (*Ananas comosus* [L.] Merr., family Bromeliaceae) is a plant native to Central America. Pineapple fruit is the most common part of plant used in both, nutrition and traditional medicine, but other parts of the plant also have certain healing properties. In our study peel and crown leaves of the fruit were extracted with absolute methanol, 80% methanol and 60% methanol in 1:10 ratio. The juice was prepared by crushing of the fruit pulp in a blender and filtering. Total phenolic content (TPC) was determined using Folin-Ciocalteu method and the antioxidant capacity was estimated in 1,1-diphenyl-2-picrylhydrazyl (DPPH) system. The highest TPC was determined in the 60% methanol leaf extract (25.94±3.54 mg GAe/g) while the lowest TPC was detected in pineapple juice (0.79±0.07 mg GAe/g). Pineapple fruit peel and crown extract in absolute methanol showed the highest antioxidant capacity (IC_{50} = 1.745±0.046 mg/ml), while the lowest antioxidant capacity was estimated in pineapple juice (IC_{50} = 88±2.09 mg/ml). The significant amount of polyphenols in pineapple fruit extracts and juice indicates their high antioxidant activity, thus these extracts and juice have a potential to be used in medicine prevention and treatment of various diseases. It is, however, necessary to perform additional *in vivo* and *in vitro* studies in order to verify pharmaceutical effects of these extracts and juice, and to examine their possible toxicity.

Key words: *Ananas comosus, polyphenols, juice, antioxidant capacity*

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1. INTRODUCTION

In recent years, the attention of scientists has been focused on the role of oxidative stress in development of many diseases. Free radicals generation is considered to be the main cause of oxidative stress, which further leads to damage of lipids, proteins and nucleic acids. This can cause development of many degenerative diseases, such as cardiovascular and nervous system disorders, cataract, arthritis, immune system malfunction, different forms of inflammation and many malignant diseases. Antioxidants, which can inhibit or delay substance oxidation in chain reactions could be significant for prevention of these diseases conditions (Bjorklund and Chirumbolo, 2017; Ćujić et al., 2013; Lobo et al., 2010; Pisoschi and Pop, 2015). Large amounts of antioxidants could be found in different kind of fruit and vegetables, and a diet rich in antioxidants could significantly reduce the occurrence of mentioned diseases (Leong and Shui, 2002; Liu, 2003; Slavin and Lloyd, 2012).

Pineapple (*Ananas comosus* [L.] Merr., family Bromeliaceae) is a plant species that originates from Central America but it is grown in many other places, mostly in Thailand, Philippines, China, Brazil, and India. Although fruit is the most used part of plant, other parts (root, rhizome, leaf, cortex, and stem) also have various nutritional and medicinal properties. Leaves, stem, and pineapple fruit, as well as their extracts, have an important role in traditional medicine as good antioxidants, antidiabetics, vermicides, emmenagogues, and hypolipemic agents. They are also used in different stadiums of wound healing and as an anti-edematous and anti-inflammatory agents in healing of soft tissue injuries, as well as in the treatment of arthritis, hematoma, and necrotic tissue (Bartholomew et al., 2003; Dutta and Bhattacharyya, 2013; Saxena and Panjwani, 2014; Xie et al., 2006; 2005).

Pineapple fruit is rich in vitamins A and C, flavonoids, tannins, and other polyphenolic compounds, organic acids, and soluble monosaccharides and disaccharides (up to 15%). Carotenoids are the source of characteristic fruit color, while the flavor comes from a very complex mix of substances where oxygenated aliphatic compounds have the main role. Stem and

full-grown fruit contain proteolytic enzyme, bromelain, which belongs to the group of endopeptidases (Bruneton, 1999; Difonzo et al., 2019).
The aim of this research was to evaluate the antioxidant activity of different methanol extracts derived from various parts of the pineapple fruit, as well as their phenolic content.

2. MATERIALS AND METHODS

2.1. Plant material

A pineapple fruit originated from Colombia, has been bought at the local market. Three different parts of fruit were separated for further extraction. The waxy leaves that are located at the top of the fruit will be referred to as crown leaves. The outer part of the fruit peel, which includes the flesh of the fruit up to the 1 cm deep, associated with the tissue which surrounds the ovaries, will be referred to as pericarp. The inner part of the fruit flash, from pericarp to central stem, including the central stem, will be referred to as pulp. The pericarp and crown leaves have been separated, chopped and dried in dark and well - ventilated area. The remaining part of the fruit has been blended and filtered through a thin layer of gauze in order to separate the pulp from clear juice. The pulp has been dried in dark and well-ventilated area. Dry plant material has been stored in paper bags and used for extraction.

2.2. Ultrasound extraction

Pericarp, peel and crown leaves and pulp extracts have been obtained by the ultrasound-assisted extraction method with absolute, 80% and 60% methanol in 1:10 ratio. The plant material has been extracted in the ultrasound bath for 40 minutes. After filtration, the obtained liquid extract was dried under vacuum apparatus (Ika-Werke, Germany). All extracts were kept in well–sealed glass bottles at 4 °C until analysis.

2.3. Determination of total polyphenolics

Total phenolic contents have been determined by Hagerman et al. (2000) colorimetric method. The method is based on the fact that phenols are reducing agents and includes the use of commercially available reduced Folin–Ciocalteu reagent that must be diluted with an equal amount of distilled water prior to analysis. The results are expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). In order to express total phenolic content, it was necessary to construct a calibration curve using different dilutions of gallic acid which were also mixed with distilled water, Folin–Ciocalteu reagent, and 20% solution of Na_2CO_3 x $10H_2O$. The absorbances were also measured at 725 nm and the curve was constructed according to gallic acid solution concentration and measured extinction ratio.

2.4. Determination of antioxidant activity using DPPH assay

Antioxidant activity presents the ability of a certain substance to donate hydrogen atom or electron. This ability of pineapple extracts was measured by following the change in color of ethanol solution of 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$) from purple to yellow, according to the method which has been described by Cuendet et al. (1997). Radical scavenging ability of $DPPH^{\bullet}$ radical was calculated by following equation:

$$\text{Inhibition of DPPH radical (\%)} = \frac{A_{control} - A_{sample}}{A_{sample}} \times 100\%$$

where $A_{control}$ is the absorbance of control (without test sample) and A_{sample} is the absorbance of the test sample at different concentrations. Curves that show the relation among extract concentrations and their inhibition percentages have been constructed and IC_{50} values (extract concentration that neutralizes 50% of free radicals)have been calculated. Antioxidant capacity has been compared to one of ascorbic acid, a substance with high antioxidant capacity.

2.5. Statistical analyses

All results have been expressed as the average of 3 parallel measurements±SD except for extracts yield. The results have been analyzed in SPSS (SPSS 20.0) with ANOVA analysis system and statistically significant difference of at least 95% ($P<0.05$) among extracts has been determined by Duncan test.

3. RESULTS AND DISCUSSION

The highest amount of total phenols was detected in the extract of leaves that surround the pineapple fruit, which was extracted by 60% methanol (25.94±3.54 mg GAE/g), fewer phenols were detected in pulp that was extracted by 80% methanol (22.99±2.36 mg GAE/g) and leaves extracted by absolute methanol (22.17±1.59 mg GAE/g), while the smallest quantity of phenols was detected in pineapple fruit juice (0.79±0.07 mg GAE/g). The results are given in Table 1.
Significant antioxidant capacity of all extracts has been proven and the highest antioxidant activity was detected in pericarp extract that was prepared with absolute methanol and its IC_{50} value 1.74±0.05 mg/mlmL. The lowest antioxidant activity was detected in pineapple juice, IC_{50} = 88.00±2.09 mg/mL. That was expected since the lowest amount of total phenols was detected in pineapple juice. The results are given in Table 1.
Tropical and subtropical fruits have a significant role in traditional medicine as a remedy for coughing, intestinal bleeding, diarrhea and many other medical conditions (Leong and Shui, 2002; Siddiq, 2012). Pineapple fruit can be used to relieve the feeling of tightening that occurs with a sore throat or to reduce sea-sickness. Hence, it is assumed that tropical and subtropical fruits can have an important role in the prevention of the occurrence and development of degenerative diseases Leong and Shui (2002); Bartholomew et al. (2003).
Many factors such as extraction method, chemical structure of polyphenols, particles size, pH value of solvent, solid to solvent ratio, extraction temperature and presence of compounds that can interact with polyphenols may have an impact on polyphenol compounds extraction from plant material. Extraction of compounds from plant material is performed in two steps. First step is swelling caused by osmotic forces. During the second step, soluble compounds are extracted and insoluble compounds hydrolyze, dissolve and diffuse (it is happening both on the surface and inside of the phase) (Jovanovic et al., 2017; Khoddami et al., 2013).
Alcohols as extraction solvents violate the integrity of the cell membrane which further leads to active compound extraction. Alcohols also have the ability to inactivate enzymes such as polyphenol oxidase, which leads to an increase in the extracted substance stability. Thus, alcohols have an advantage compared to other solvents. Polyphenols are soluble in polar solvents and with higher solvent polarity the amount of isolated polyphenols increases Mandić (2007).
Researchers have shown that significantly higher levels of total polyphenols can be found in dried pineapple fruit parts, compared to the fresh ones. It is considered that this is a consequence of polyphenol extraction from plant cell vacuoles (da Silva et al., 2013).
Mhatre et al. (2009) confirmed that the highest antioxidant activity in DPPH system belongs to the methanol extracts of pineapple fruit (1933.0±9.1% equivalent of ascorbic acid) compared to ethyl acetate and water extracts (1051.8±19.6% and 612.1±12.4% equivalent of ascorbic acid).

Table 1. Extraction yields and total phenolic content in juice and methanol extracts of pulp, crown leaves and pericarp of pineapple fruit

Solvent	Plant part	Extraction yield [%]	Total phenolic content [mg GAE/g]	DPPH (IC_{50}) [mg/mL]
MeOH 100%	Pulp	24.24	16.15±0.14 ab	2.90±0.10 a
	Leaves	2.55	22.17±1.59 a-c	1.91±0.06 a
	Pericarp	8.07	17.57±1.72 a	1.74±0.05 a
MeOH 80%	Pulp	24.73	22.99±2.36 bc	8.19±0.66 c
	Leaves	2.40	20.24±1.04 ab	89.66±0.93 e
	Pericarp	10.78	18.86±0.13 ab	7.19±0.68 c
MeOH 60%	Pulp	24.64	20.05±0.34 ab	7.56±0.66 c
	Leaves	2.10	25.94±3.54 c	2.77±0.21 a
	Pericarp	9.37	17.56±3.22 a	11.79±1.07 d
Juice	Pulp	-	0.79±0.07 d	88.00±2.09 e
Control (Ascorbic acid)				0.0047±0.0003 b

[a]Values with the same letter (a-d) in each column showed no statistically significant difference ($P < 0.05$); Statistical analysis was based on a one-way ANOVA and Duncan's test.

Antioxidant capacity of tropical and subtropical fruits is usually attributed to high levels of L-ascorbic acid. However, many researchers proved that, when it comes to antioxidant capacity, the main role belongs to the synergism of many active compounds meaning that, aside from L-ascorbic acid, there are other substances that can act as antioxidants themselves, as well as substances that have no antioxidant capacity but may intensify antioxidant capacity of L-ascorbic acid (Leong and Shui, 2002; Ferreira et al., 2016).

It has been proven that water-ethanolic extracts made of different pineapple parts have cardioprotective effects which are achieved through lowering the levels of cholesterol, triglycerides, LDL, and VLDL particles, as well as levels of aspartate transaminase and alanine transaminase in serum with the simultaneous increase of HDL particles levels Fleming (2004). The methanolic extract of pineapple crust has shown modulatory activity on alcohol-induced lipid peroxidation in the liver causing the reduction of hepatic biomarkers in rat plasma. These effects are attributed to high levels of polyphenolic compounds that have been isolated from extracts. The antioxidant capacity is achieved through several different mechanisms: free radical scavenging, oxidative enzymes inhibition, catalase activity increase, and metal chelation (Okafor et al., 2011). Research has shown that pineapple crust extract has protective effects on brain tissue due to its ability to prevent an alcohol-induced change in phospholipids and lipid peroxidation (Erukainure et al., 2011). Pineapple extracts have shown a significant effect in reducing the inflammation in the gastrointestinal tract and as a potential cure in the treatment of Crohn's disease and ulcerous colitis Onken et al. (2008).

In everyday pineapple fruit usage over 40% of the fruit is turned into organic waste. However, new researches show that pericarp and peel and crown leaves of the fruit contain significant amounts of antioxidants, possibly even larger amounts than the edible part of the fruit (Leong and Shui, 2002).

4. CONCLUSION

High levels of total polyphenols in the methanol extracts and the pineapple juice, as well as a consequently significant antioxidant activity, grant all parts of the pineapple fruit a potential use in modern medicine. However, it is necessary to conduct further *in vitro* and *in vivo* analysis in order to confirm pharmacological effects of these extracts and juice and their eventual toxicity.

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REFERENCES

Bartholomew, D. P., Paull, R. E. and Rohrbach, K. G. (eds) (2003). *The pineapple: botany, production and uses*, CABI, Wallingford.

Bjorklund, G. and Chirumbolo, S. (2017). Role of oxidative stress and antioxidants in daily nutrition and human health, *Nutrition* **33**: 311–321.

Bruneton, J. (1999). *Pharmacognosy, Phytochemistry, Medicinal Plants*, Intercept. Google-Books-ID: BaZrQgAACAAJ.

Cuendet, M., Hostettmann, K., Potterat, O. and Dyatmiko, W. (1997). Iridoid Glucosides with Free Radical Scavenging Properties fromFagraea blumei, *Helvetica Chimica Acta* **80**(4): 1144–1152.

Ćujić, N., Kundaković, T. and Šavikin, K. (2013). Anthocyanins – chemistry and biological activity, *Lekovite Sirovine* **33**(0): 19–37.

da Silva, D. I., Nogueira, G. D., Duzzioni, A. G. and Barrozo, M. A. (2013). Changes of antioxidant constituents in pineapple (Ananas comosus) residue during drying process, *Industrial Crops and Products* **50**: 557–562.

Difonzo, G., Vollmer, K., Caponio, F., Pasqualone, A., Carle, R. and Steingass, C. (2019). Characterisation and classification of pineapple (Ananas comosus [L.] Merr.) juice from pulp and peel, *Food Control* **96**: 260–270.

Dutta, S. and Bhattacharyya, D. (2013). Enzymatic, antimicrobial and toxicity studies of the aqueous extract of Ananas comosus (pineapple) crown leaf, *Journal of Ethnopharmacology* **150**(2): 451–457.

Erukainure, O., Ajiboye, J., Adejobi, R., Okafor, O., Kosoko, S. and Owolabi, F. (2011). Effect of pineapple peel extract on total phospholipids and lipid peroxidation in brain tissues of rats, *Asian Pacific Journal of Tropical Medicine* **4**(3): 182–184.

Ferreira, E. A., Siqueira, H. E., Boas, E. V. V., Hermes, V. S. and Rios, A. D. O. (2016). Bioactive compounds and antioxidant activity of pineapple fruit of different cultivars, *Revista Brasileira de Fruticultura* **38**(3).

Fleming, T. (ed.) (2004). *PDR for Herbal Medicines*, 3rd edn, Thomson Healthcare.

Hagerman, A., Harvey-Mueller, I. and Makker, H. (2000). Quantification of tannins in the foliage-a, *Laboratory Manual*, FAO/IAEA, Vienna.

Jovanovic, A., Petrovic, P., Đordjevic, V., Zdunic, G., Savikin, K. and Bugarski, B. (2017). Polyphenols extraction from plant sources, *Lekovite sirovine* **37**: 45–49.

Khoddami, A., Wilkes, M. and Roberts, T. (2013). Techniques for Analysis of Plant Phenolic Compounds, *Molecules* **18**(2): 2328–2375.

Leong, L. and Shui, G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets, *Food Chemistry* **76**(1): 69–75.

Liu, R. H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals, *The American Journal of Clinical Nutrition* **78**(3): 517S–520S.

Lobo, V., Patil, A., Phatak, A. and Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health, *Pharmacognosy Reviews* **4**(8): 118.

Mandić, A. (2007). *Antioxidant properties of white grape varieties extracts*, PhD thesis, Faculty of Technology, University of Novi Sad.

Mhatre, M., Tilak-Jain, J., De, S. and Devasagayam, T. P. (2009). Evaluation of the antioxidant activity of non-transformed and transformed pineapple: a comparative study., *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* **47**(11): 2696–2702.

Okafor, O., Erukainure, O., Ajiboye, J., Adejobi, R., Owolabi, F. and Kosoko, S. (2011). Modulatory effect of pineapple peel extract on lipid peroxidation, catalase activity and hepatic biomarker levels in blood plasma of alcohol–induced oxidative stressed rats, *Asian Pacific Journal of Tropical Biomedicine* **1**(1): 12–14.

Onken, J. E., Greer, P. K., Calingaert, B. and Hale, L. P. (2008). Bromelain treatment decreases secretion of pro-inflammatory cytokines and chemokines by colon biopsies in vitro, *Clinical Immunology* **126**(3): 345–352.

Pisoschi, A. M. and Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review, *European Journal of Medicinal Chemistry* **97**: 55–74.

Saxena, P. and Panjwani, D. (2014). Cardioprotective potential of hydro-alcoholic fruit extract of Ananas comosus against isoproterenol induced myocardial infraction in Wistar Albino rats., *Journal of Acute Disease* **3**(3): 228–234.

Siddiq, M. (ed.) (2012). *Tropical and Subtropical Fruits: Postharvest Physiology, Processing and Packaging*, Wiley-Blackwell, Oxford, UK.

Slavin, J. L. and Lloyd, B. (2012). Health Benefits of Fruits and Vegetables, *Advances in Nutrition* **3**(4): 506–516.

Xie, W., Wang, W., Su, H., Xing, D., Pan, Y. and Du, L. (2006). Effect of ethanolic extracts of Ananas comosus L. leaves on insulin sensitivity in rats and HepG2, *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **143**(4): 429–435.

Xie, W., Xing, D., Sun, H., Wang, W., Ding, Y. and Du, L. (2005). The Effects of *Ananas comosus* L. Leaves on Diabetic-Dyslipidemic Rats Induced by Alloxan and a High-Fat/High-Cholesterol Diet, *The American Journal of Chinese Medicine* **33**(01): 95–105.