

THE ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACTS FROM BLACK PEPPER (*PIPER NIGRUM* L.) FRUITS OBTAINED BY DIFFERENT EXTRACTION TECHNIQUES

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Black pepper (*Piper nigrum* L.) is a flowering plant, originates from the evergreen forests of southwestern India and Southeast Asia. Black pepper is mainly used as a spice, to enhance food flavor as well as in food preserving, but has also been explored for its biological properties and its bioactive compounds. The aim of this study was to determine the antioxidant potential of black pepper fruits ethanolic extracts (BPFEE) obtained by different extraction techniques (maceration-M, reflux extraction-RE, ultrasonic extraction-UE and Soxhlet extraction-SE), using several antioxidant assays (DPPH, ABTS, FIC, FRAP and Ferricyanide method). Total phenols and total flavonoids content was determined spectrophotometrically by the method of Folin-Ciocalteu and AlCl_3 , respectively. BPFEE obtained by RE at a boiling temperature was found to possess the highest antioxidant activity including the scavenging of DPPH and ABTS radicals (EC_{50} values determined by DPPH and ABTS test were 0.112 ± 0.0012 mg/ml and 1.010 ± 0.002 mg/ml, respectively). The same extract was reached the maximum iron ions chelating ability determined by FIC test (EC_{50} value was 1.146 ± 0.0155 mg/ml), and the highest reducing effect (FRAP value was 67.82 ± 0.08 mgFe²⁺/g d.e.). The best ability to reduce the ferric-ferricyanide (Fe^{+3}) complex has showed BPFEE obtained by UE at room temperature with value of 31.2 ± 0.22 mgGAE/g of dry extract. The BPFEE obtained by UE at a room temperature resulted in the highest phenolic content (85.64 mgGAE/g d.e.), while the highest flavonoid content was observed for BPFEE obtained by RE at a boiling temperature (97.56 mgRE/g d.e.).

A significant correlation was found between the antioxidant activity of extracts and their total phenolic and flavonoid content. Presented results show that there is a space for further investigations into the isolation and identification of responsible antioxidant components and their mechanism of action to better understand their ability to be used as a safer alternative antioxidant agents.

INTRODUCTION

- ❑ **Black pepper** (*Piper nigrum* L.) is a flowering plant from family Piperaceae;
- ❑ The fruits, which are called peppercorns, are mostly used as a spice, because of their aromatic odour, the hot, biting, and very pungent taste.
- ❑ Black pepper is also used in many traditional medicines and in cosmetics because of its various biological properties and very valuable compounds.
- ❑ Black pepper contains up to 3% essential oil that has the aromatic flavour. The characteristic pungency is principally derived from the alkaloid piperine, although the fruit also contains other alkaloids such as chavicine, piperidine, and piperettine.



Figure 1 Black pepper (*Piper nigrum* L.) plant (a) with peppercorns (b)
(Wikipedia contributors. "Black pepper." Wikipedia, The Free Encyclopedia. Wikipedia, The Free Encyclopedia, 10 May. 2021. Web. 11 May. 2021.)



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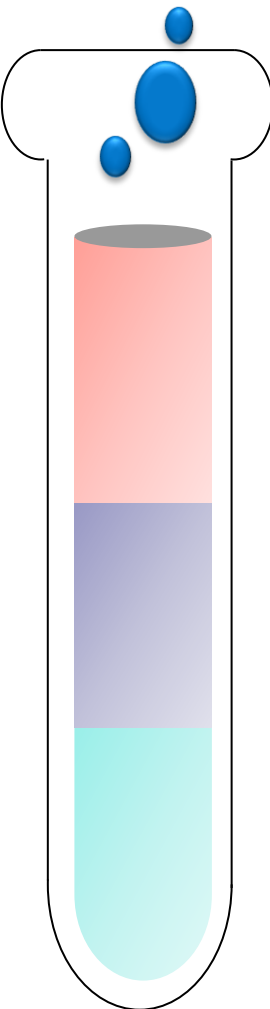


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AIM OF THE STUDY

The aim of this study was to determine the antioxidant potential of black pepper fruits ethanolic extracts (BPFEE) obtained by different extraction techniques (maceration-M, reflux extraction-RE, ultrasonic extraction-UE and Soxhlet extraction-SE), using several antioxidant assays (DPPH, ABTS, FIC, FRAP and Ferricyanide method).

METHODS

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- ❑ In this study were used different methods of extraction at solvomodule 1:10 m/v, with 70% EtOH:
 - maceration (at a room temperature, 180 min);
 - reflux extraction (40°C, 50°C and at boiling temperature, 180 min);
 - ultrasonic extraction (at room temperature, 60 min);
 - Soxhlet extraction (240 min).

 - ❑ Total phenols and flavonoids content was determined spectrophotometrically by the method of Folin-Ciocalteu and AlCl_3 , respectively.

 - ❑ The antioxidant potential of obtained extracts was determined by several antioxidant assays:
 - DPPH assay;
 - ABTS assay;
 - FIC assay;
 - FRAP assay;
 - Ferricyanide method.

RESULTS AND DISCUSSION

	Total phenols mgGAE/g d.e.	Total flavonoids mgRE/g d.e.	EC ₅₀ , DPPH mg/ml ± sd.	EC ₅₀ , ABTS mg/ml ± sd.	EC ₅₀ , FIC mg/ml ± sd.	mgEFe ²⁺ /g d.e.± sd., FRAP	mgGAE/g d.e. ± sd. Red.power (Fe ³⁺ - Fe ²⁺)
Time of incubation	90 min incubation	45 min incubation	20 min incubation	6 min incubation	10 min incubation	30 min incubation	20+30 min incubation
Maceration	69.54	59.66	0.152 ± 0.001	1.73 ± 0.008	1.353 ± 0.0006	64.67 ± 0.35	25.1 ± 0.22
Reflux extraction 40°C	76.11	59.80	0.138 ± 0.0007	1.63 ± 0.012	1.319 ± 0.017	65.13 ± 0.06	25.97 ± 0.22
Reflux extraction 50°C	78.61	60.07	0.133 ± 0.0003	1.110 ± 0.007	1.197 ± 0.0146	66.64 ± 0.05	29.24 ± 0.22
Reflux extracton (boiling temperature)	79.29	97.56	0.112 ± 0.0012	1.010 ± 0.002	1.146 ± 0.0155	67.82 ± 0.08	27.28 ± 0.22
Ultrasonic extraction	85.64	73.15	0.142 ± 0.0004	1.010 ± 0.005	1.646 ± 0.0046	66.64 ± 0.05	31.2 ± 0.22
Soxhlet extraction	74.75	53.26	0.120 ± 0.0007	1.110 ± 0.004	1.327 ± 0.0099	63.72 ± 0.05	22.27 ± 0.22

*GAE = Gallic acid equivalent; * RE = Rutin equivalent ; * d.e. = dry extract

RESULTS AND DISCUSSION



- A significant correlation was found between the antioxidant activity of extracts and their total phenolic and flavonoid content.
- BPFEE obtained by reflux extraction at boiling temperature showed the best antioxidant potential according to DPPH, ABTS, FIC, FRAP test. The same extract showed the highest content of total flavonoids.
- BPFEE obtained by ultrasonic extraction at room temperature also showed excellent antioxidant potential, and at the same time the highest content of phenols. The effect of ultrasound has a positive effect on the duration of the process of extraction of total phenols from the black pepper fruit.

CONCLUSION

- Based on the obtained results, it can be concluded that black pepper is a potential source of natural antioxidants, and there is a space for further investigations into the isolation and identification of responsible antioxidant components and their mechanism of action to better understand their ability to be used as a safer alternative antioxidant agents.



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ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Program for financing scientific research work, number 451-03-9/2021-14/200133.