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RESEARCH ARTICLE

THE INFLUENCE OF EXTRACTION METHOD AND EXTRACTION TIME ON PHENOLIC COMPOUNDS CONTENT AND ANTIOXIDANT ACTIVITY OF *PALISOTA HIRSUTA* ROOTS (*K.*SCHUM, COMMELINACEAE)

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ABSTRACT

The influences of different extraction methods and the extraction time of total phenols, flavonoids, condensed tannins, and the antioxidant activity of the ethanolic extracts obtained from the roots of *Palisota hirsuta* were studied. The extractions were performed by maceration, decoction and Soxhlet method, with absolute ethanol every 30 min (30 min, 1h,1h30 min, 2h and 2h 30). For each extract it was determined the total phenolic content (TPC), the total flavonoid content (TFC), the total condensed tannins (TC), the ability to scavenge DPPH, and Fe^{3+} reducing power. Soxhlet extraction showed the highest yield extraction. Maceration method led to the highest concentration of phenolics and flavonoids, and also the highest DPPH scavenging activity. However, the highest concentration of condensed tannin and the highest Fe^{3+} reducing power were obtained by Soxhlet used. Beside in general, the TPC, TC, TFC and antioxidant activity increased when extraction time was increased from 30 min to 2h, but their decreased after 1h30 min for using as decoction and Soxhlet methods.

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INTRODUCTION

Plants materials represent a source of active ingredients known for long times ago by their traditional used for medical purposes (Liu *et al*., 2015). Medicinal plants produce a large variety of secondary metabolites, which can be divided into three chemically distinctive groups namely: Terpenes, phenolic compounds and nitrogen containing compounds (Mohiuddin, 2019). Secondary metabolites have been found to have a broad range of therapeutic properties, including antioxidant activities (Shashirekha, 2015). The researchers suggested that compounds with antioxidant activity are able to remove the excess of free radicals in the body, prevent or cure diseases caused by oxidative stress such as cardiovascular diseases, inflammation, atherosclerosis, cancer, degenerative diseases such as Alzheimer and Parkinson (Shashirekha, 2015; Predescu *et al*., 2016). Plant secondary metabolites are complex compounds with undeniable biological, physical, and chemical properties. Some of the important steps when working with secondary metabolites are their extraction and isolation of imbedded compounds. Several studies have shown the influence of different parameters on the extraction of bioactive compounds; the temperature, the nature of the extraction solvent, the solid-to-solvent ratio, the extraction time and the extraction method used (Liorach *et al*., 2004). Ivory Coast is well known for exuberance and variety of medical plants (Okpekon, 2014). One of the most popular medicinal plants is *Palisota hirsute* (Commelinaceae). Different parts of the plant are used in West Africa folkloric medicine for the treatment of pain and inflammatory conditions (Bouquet, 1974).

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The main objective of our research work was to analyze the influence of the methods performed and the conditions of the extraction, especially on the total phenolics, flavonoids, tannins content, and the antioxidant activity of the *Palisota hirsuta* roots.

MATERIALS AND METHODS

Plant material: *Palisota hirsuta* roots were collected at Nangui Abrogoua University (Abidjan- Côte d'Ivoire). Collected samples were cleaned and dried under conditioning air at room temperature at 20°C for fifteen days and finally grinded into fine powder.

Extractions: The extractions were carried out by maceration, decoction and Soxhlet methods.

Maceration: 5 g of roots powder of *Palisota hirsuta* was macerated in 40 mL absolute ethanol at room temperature for 30 min (M1), 1h (M2), 1h 30 min (M3) et 2 h (M4). Extract was filtered and evaporated with a rotary evaporator at 35°C.Thus, the extract of the roots was prepared and kept for future analyzes.

Decoction:5 g of roots powder of *Palisota hirsute* was heated in 40 mLabsolute ethanol at 78,3°C, for 30 min (D1), 1h (D2), 1h 30 min (D3) et 2h (D4). Extract was filtered and evaporated with a rotary evaporator at 35°C.Then, the extract of the roots was prepared and kept for our future experiments.

Soxhlet extraction:5 g of roots powder of *Palisota hirsute* was placed in a porous bag or in a thimble made with filter paper or cellulose. In the thimble chamber of the Soxhlet apparatus; the solvent used for the extraction was absolute ethanol, the solvent was then heated at 78,3°C in a flat bottom flask for 1h (S1), 1h 30 min (S2), 2h (S3) et 2h 30min (S4).The extracts were prepared, evaporated with a rotary evaporator at 35°C and kept for the experiments.

Qualitative test: The phytochemical screening using thin-layer chromatography (TLC) was carried out according to the analytical procedure used in literature (8, 9). TLC chromatogram of the extract was eluted in $CH_3CO_2C_2H_5$ / CH_3COOH / H_2O / $CHCl_3$ (2.5:1:0.1:1.5, v/v/v/v). Furthermore, in order to be able to precise their nature; specific reagents used for flavonoids were ($AlCl_3$,1%, w/v) and ammonia (NH_3). For coumarins; KOH 5%, w/v and $(CH_3COO)_2$ Pb 5%, w/v; NH_3 were used. However, for tannins and phenolic acids, it was used ($FeCl_3$, 2%, w/v); and NH_3 for anthocyans.

Quantitative test

Determination of total phenolic: Total phenolic content (TPC) was determined according to the Folin-Ciocalteucolorimetric method (Heilerová *et al*., 2003; Konan, 2010). The sample (1 mL) and 1.5 mL of sodium carbonate (17%, w/v) were added to 0.5 mL of Folin-Ciocalteu reagent (0.5N). After 30 min of reaction at room temperature, the absorbance was measure at 760 nm. The total phenol concentration was calculated from gallic acid calibration curve (0-1000 µg/mL) and expressed in µg of gallic acid equivalent/g of dry weight (µg EAG/g MS).

Dosage of total Flavonoids: The total flavonoid content (TFC) was evaluated using NEU reagent **(**Hariri, 1991**).** 2 mL of the sample were added to 100 µL of NEU reagent and the absorbance was measured at 404 nm.The percentage of TFC was expressed as µg EAG/g MS.

Determination of condensed tannins: Condensed tannins content (TC) was determined by the method using catech in as a reference compound (Heim *et al*., 2002). A volume of 400 µL of extract was added to 3 mL of vanillin solution (4% in methanol) and 1.5 mL of concentrated hydrochloric acid. After 15 min of incubation the absorbance was read at 500 nm. The concentration of total condensed tannin was calculated from catechin calibration curve (0-300µg/mL) and was expressed as µg catechin equivalent/g of dry weight (µg C/g MS).

Determination of the antioxidant activity of the extract

Determination of DPPH free radical scavenging activity: The DPPH radical-scavenging activity was determined on the different extracts (Espin *et al*., 2000). 0.4 mL of various concentrations of the ethanolic samples (0.128; 0.0357;0.0014; 0.001 and 0.00071 mg/mL), were added to 1.2 ml of 0.03 mg/mL methanolic solution of DPPH. At room temperature and every 3 min; the absorbance was measured and read at 517 nm. The percentage of discolored DPPH (PR) and the efficiency index (IE)of the sample was calculated according to the following equation (2) et (3):

$$PR(\%) = \left(1 - \frac{A_e}{A_b}\right) \times 100 \quad (2)$$

$$IE = \frac{CI_{50,t}}{(DPPH)t=0} \quad (3)$$

The ferric reducing antioxidant power assay (FRAP) of each standard solution was measured according to a modified protocol developed (Benzie, 1996; Gong *et al*., 2016). To prepare the FRAP reagent, a mixture of 0.1 M of acetate buffer (pH 3.6), 10 mM TPTZ, and (20 mM) ferric chloride (10:1: 1, v/v/v) was used.100 µL of extract (0.25 mg/mL) were added to 3 mL of FRA Preagent. After 4 min the absorbance was read at 593 nm at room temperature. Trolox solution at various concentrations (0.187; 0.375; 0.75 et 1.5 mM) was used to perform the calibration curves. The reducing antioxidant power were expressed as mM of Trolox equivalent/ g of dry weight (mM ET/g MS).

RESULTS AND DISCUSSION

Extraction yields: The results showed a significant increase of the extraction yield when the extraction time was increased. Generally, it was noticed that the maximum extraction yield was achieved at 1h 30 min. After this peak, it was observed a slight and not significant increase of the extraction yield throughout the experiment. However, higher extraction yields were registered using Soxhlet method (S1, S2, S3 and S4) for solid-to-solvent ratio of 1/30 (w/v),compared to the values obtained after the decoction and the maceration methods with ratio of 1/8 (w/v) (Table 1).The extraction time and solid to liquid ratio (S/L) are significant parameters which can drastically influence the content of bioactive compounds in extracts. Furthermore, the extraction yield depends on the temperature.

Table 1. Extraction yields

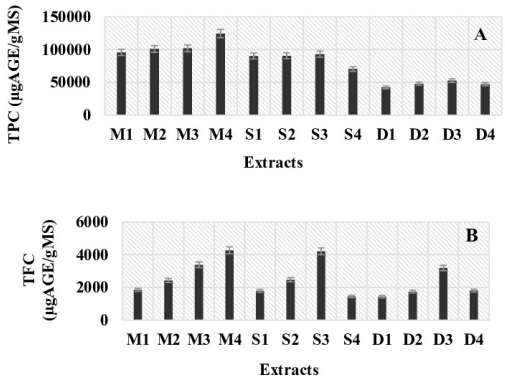
Yields (%)	Maceration				Decoction				Soxhlet			
	M1	M2	M3	M4	D1	D2	D3	D4	S1	S2	S3	S4
	1.4	1.6	2	2.1	1.8	2.4	2.8	2.9	5.4	7.2	7.4	7.6

M1-4: extracts obtained by maceration at different time; S1-4: extracts obtained by Soxhlet at different time;D1-4: extracts obtained by decoction at different time.

Table 2: Phytocompounds detected in *Palisota hirsuta* roots by TLC

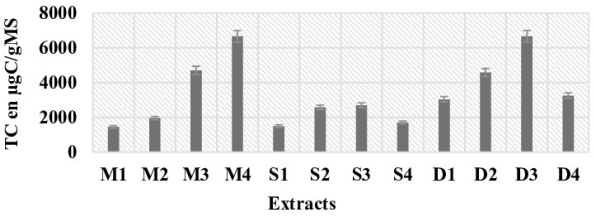
Extrait	Rf, (Color), Possible phytocompound
Maceration (M1, M2, M3, M4)	0.10 (G^a-YG^c) Fl/Cou ; 0.125 (B^a) Fl ; 0.14 (G^b) Fl ; 0.17 (B^c, B^d) Cou ; 0.24 (Y^a) Fl ; 0.35 (Y^b) Fl ; 0.75 (YG^a) Fl ; 0.9 (B^a) Fl
Decoction (D1, D2, D3, D4)	0.10 (G^a-YG^c) Fl/Cou ; 0.125 (B^a) Fl ; 0.14 (G^b) Fl ; 0.17 (B^c, B^d) Cou ; 0.22 (B^b-B^d) Ant/Cou ; 0.24 (Y^a) Fl ; 0.35 (Y^b) Fl ; 0.48 (B^c) ; 0.59 (B^c) Cou ; Cou ; 0.68 (P^b) Ant ; 0.72 (B^c-G^d) Cou ; 0.75 (YG^a) Fl ; 0.9(B^a-V^b) Fl ; 0.79 (Br^a-B^d) Fl/Cou ; 0.8 (P^b) Ant ; 0.82 (DG^c) Cou ; 0.87 (DG^c) Cou
Soxhlet (S2, S3, S4, S5)	0.10 (G^a-YG^c) Fl/Cou ; 0.125 (B^a) Fl ; 0.14 (G^b) Fl ; 0.17 (B^c-B^d) Cou ; 0.22 (B^b-B^d) Ant/Cou ; 0.24 (Y^a) Fl ; 0.35 (Y^b) Fl ; 0.48 (B^c) Cou ; 0.59 (B^c) Cou ; 0.68 (P^b) Ant ; 0.72 (B^c-G^d) Cou ; 0.75 (YG^a) ; 0.9(B^a-G^b) Fl ; 0.79 (Br^a-B^d) Fl/Cou ; 0.80 (P^b-R^e) Ant/AcP ; 0.82 (DG^c-G^e) Cou/Tn ; 0.87(DG^c) Cou

G : green ; B : blue;Y: yellow ; Br : brown ; DG : dark green ; GY : grey ; YG : yellow green ; P:purple ; R : red ; a : $AlCl_3$ test; b : NH_3 test ; c : KOH test ; d : acetate of lead test ; e : $FeCl_3$test; Fl : Flavonoids ; Cou : Coumarins ; Ant : Anthocyans ; AcP : phenolic acids ; Tn : Tannins. M1-4: extracts obtained by maceration at different time; S1-4: extracts obtained by Soxhlet at different time; D1-4: extracts obtained by decoction at different time.



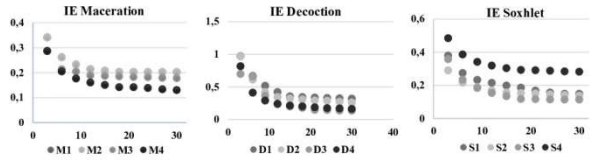
M1-4: Extracts obtained by maceration at differnt time; S1-4: extracts obtained by Soxhlet at different time; D1-4: extracts obtained by decoction at different time

Figure 1. Total phenolic (a) flavonoid (b) content of Palisota hirsuta roo



M 1-4: extracts obtained by maceration at different time; S1-4: extracts obtained by Soxhlet at different time; D1-4: extracts obtained by decoction at different time

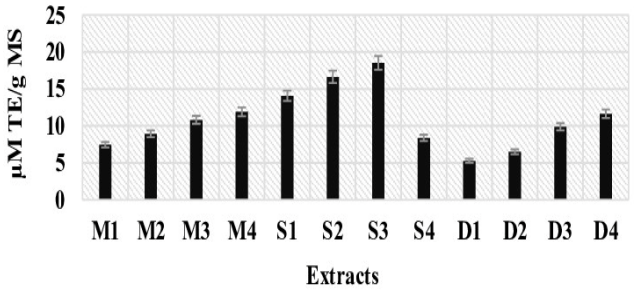
Figure 2. Condensed tannins content of Palisota hiruta roots



M 1-4: extracts obtained by maceration at different time; S1-4: extracts obtained by Soxhlet at different time; D1-4: extracts obtained by decoction at different time.

Figure 6. Evaluation of antioxidant activity of Palisota *hirsute* roots by DPPH test

The use of higher temperatures leads to the reduction in the viscosity of the solvent and facilitates the diffusion (Pinelo *et al*., 2017; Lafka *et al*., 2007).



M1-4: extracts obtained by maceration at different extraction, S1-4: extracts obtained by Soxhlet at different extraction, D1-4: extracts obtained by decoction at different extraction

Figure 4. Evaluation of antioxidant activity of Palisota *hirsute* roots by FRAP essay

Qualitative test: Phytochemical screening of the leaves of *Palisota hirsuta* revealed the presence of tannins, flavonoids, triterpenoids, coumarins, phytosterols and reducing sugars (Sarpong *et al*., 2016). The results of phytochemical screening in our study are reported in (Table 2).

Quantitative test: Determination of TPC and TFC: The total phenolic content (TPC) was obtained from the regression equation of gallic acid ($y = 0.0232 x + 0.0002$; $R^2 = 0.9983$) and the percentage of total flavonoid content (TFC) was calculated from TPC and expressed as µg EAG/g MS. The comparative results on TPC and TFC of the extracts depending to the extraction methods used are presented in (Figure 1). The TPC and TFC obtained after using the maceration method were higher compared to those found using the decoction method, however; intermediate values were obtained using the Soxhlet extraction. (Figure 1) shows that an increase of the temperature from 25 up to 78,3°C will decrease the TPC and TFCin the roots of *Palisota hirsuta*. These results confirm the presence of tannins, flavonoids, coumarins and phenolic acids in the roots of *Palisota hirsuta*, revealed by TLC chromatography. Similar trend was also reported by other researchers (Mahmoudi *et al*., 2013; Bourgou *et al*., 2016); they reported that TPC and TFC obtained for the extractions carried out by maceration were significantly higher compared to those obtained after using the decoction method. Additionally, Predescu reported that PT found in vegetal

materials extracted using the maceration method was significantly higher compared to the one found after the Soxhlet method (Predescu *et al*., 2016).

Determination of the condensed tannins content (TC): Condensed tannins content (TC) value was obtained from the regression equation of catech in (y = 0.004 x + 0.006) and expressed as µg Catechin/g MS. Figure 2 shows the comparative results on TC of investigated extracts based on the extraction method and the extraction time. From 30 min to 1 h 30min, TC found in the roots of *Palisota hirsuta* extracted by the decoction method was significantly higher compared to maceration and Soxhlet methods. After 2h, the lower TC content obtained after using the decoction and Soxhlet methods was due to the degradation of condensed tannins at high temperature. This quantitative test revealed the TC which did not reveal by chromatography TLC. In a study on the tannins extracted from Moroccan *Acacia mollissima* barks (Naima *et al*., 2015), it was reported that TC content obtained after using the maceration at 20°C was significantly higher compared to the infusion (60°C).

Determination of the antioxidant activity of the extract

Determination of DPP Hradical scavenging activity: Figure 3 shows the antioxidant activity expressed as efficiency index (EI) for the investigated extracts. The higher radical scavenging activity is associated with a lower IE values (good antioxidant activity) (Falleh *et al*., 2006). The ability of the extracts to scavenge DPPH radical was significantly dependent on the extraction method used and the extraction time. For the investigated extracts, the values of IE detected after using the maceration method were higher compared to those found after the decoction and Sox let methods. Increasing the extraction time from 30 min to 2h will increase the overall antioxidant activity after using the maceration, while the antioxidant activity found for the decoction and Sox let methods were decreased. Other authors were reported the antioxidant activities in aqueous extracts of *Palisota hirsute* (Maloueki, 2013). In another study on Flavonoid antioxidants, it was reported that the antioxidant activities depend on the concentration of phenolic compounds, their number, and the localization of the hydroxyl groups (Heim, 2002).

Ferric reducing antioxidant power assay (FRAP): The comparative results on Fe^{3+}reducing power of the investigated extracts depending on the extraction methods used and the extraction time are presented in Figure 4. In the present study, it was found that the reducing power increased when the extraction time was increased from 30 min to 2h for the three extraction methods performed. However, the reducing power obtained for the extractions carried out by Soxhlet method were decreased after 1h30 min. The results from this study showed that the constituents imbedded in the roots of *Palisota hirsute* have some antioxidant activities. Some authors reported that Fe^{3+}reducing power is appropriate to measure the total antioxidant capacity (Kumaran, 2007).

Conclusion

This work showed the impact of extraction method and extraction time on phenolic compounds content and antioxidant activity of *Palisota hirsuta* roots. The higher extraction yields were registered using Soxhlet method. The phytocompounds screening of the revealed the presence of severalphyto compounds. The total phenol, total flavonoids and condensed tannins were copresent with variable proportion, and, it was according to the extraction method. The extended extraction time (after 1h 30 min) during extraction under thermal induction, showed a certain thermo sensibility of phenolic compounds from roots of *Palisota hirsuta*. The antioxydant activity showed similar tendency by DPPH and FRAP essay.

Conflit of interest: None

LIST OF ABBREVIATIONS

Ab: Absorbance of control
Ae: Absorbance of sample
D1-D4: extracts obtained by decoction at different time
DPPH: 2,2-Diphenyl-1-picrylhydrazyl Radical
FRAP: Ferric Reducing Antioxidant Power
IE: Efficiency Index
M1-M4: extracts obtained by maceration at different time
PR: percentage of discolored DPPH
S/L: Solid-Liquid
S1-S4: extracts obtained by Soxhlet at different time
TC: Condensed tannins content
TFC: Total Flavonoid Content
TLC: Thin-Layer Chromatography
TPC: Total phenolic content
TPTZ: **(**2, 4,6-Tri (2-pyridyl)-s-triazine)

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