

Available online at [GSC Online Press Directory](#)

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: <https://www.gsonlinepress.com/journals/gscbps>

(RESEARCH ARTICLE)



Influence of temperature on the phytochemical composition and the antioxidant and anticariogenic activities of extracts from the husk of the fruit of *Cocos nucifera* L. (Arecaceae)

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Publication history: Received on 14 August 2020; revised on 22 August 2020; accepted on 26 August 2020

Article DOI: <https://doi.org/10.30574/gscbps.2020.12.2.0265>

Abstract

With a view to contributing to the enhancement of medicinal plants from the Ivorian flora, this work was initiated in order to study the influence of temperature on the phytochemical composition and the antioxidant and anticariogenic activities of *Cocos nucifera* L. by the study of the envelope of its fruit. For this purpose, maceration, digestion at 50°C and 70°C, and decoction were carried out to provide 4 extracts respectively TA, TB, TC, TD, which were subjected to phytochemical and anticariogenic tests. Phytochemical screening indicated the coexistence of coumarins, flavonoids, steroids, terpenes and tannins. However, this composition varies depending on the type of extraction. Then, the evaluation of the antioxidant activity vis-à-vis DPPH showed good activity of the extracts. The greatest activity was observed with the TC extract (EC₅₀ = 2.56). Finally, the study of the antibacterial potential revealed that the extracts are all bactericidal against the cariogenic strains tested.

Keywords: Phytochemistry; Temperature; *Cocos nucifera*; DPPH; *Streptococcus mutans*

1. Introduction

Over the past century, the epidemiological situation of dental caries has evolved differently around the world. According to the WHO, this infectious disease of the tooth is ranked among the top 4 major global public health scourges [1].

In order to provide a lasting curative response, investigations into plants with anti-odontalgia properties are being carried out at the Laboratoire de Chimie Bio-Organique et de Substances Naturelles (LCBOSN). This is the case with *Cocos nucifera* L. from the Arecaceae botanical family, whose root decoction is used as a mouthwash against dental pain in Côte d'Ivoire. A previous study by Kadja et al. (2020) [2] on the organs of 2 varieties of this plant demonstrated their preliminary phytochemical composition and their antioxidant potential. The work has also shown a significant antioxidant profile of the yellow envelope of *C. nucifera*.

It is in this context that the main objective of the present study is to study the influence of temperature on the phytochemical composition and the antioxidant and anticariogenic activities of the husk of the fruit of this plant.

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2. Material and methods

2.1. Materiel

2.1.1. Plant material

The plant material consists of the shell of the yellow fruit of the coconut palm. These fruits were harvested in the municipality of Abobo located in the district of Abidjan (5°26'00" north, 4°01'00" west), on May 05, 2019. The fruit husks were cut, washed, dried at 18°C for 3 weeks under air conditioning and sprayed with a blender. The powder was saved.

2.1.2. Bacterial material

The bacterial strains *Streptococcus mutans* (*S. mutans* 1142C/14 and *S. mutans* 1143C/14) used, come from the biobank of the Pasteur Institute of Côte d'Ivoire (IPCI).

2.2. Methods

2.2.1. Extraction of phytochemicals

Maceration

Using an Erlenmeyer flask, 100 g of vegetable powder was macerated in 700 mL of distilled water for 30 min. This operation was repeated 3 times with the same marc. After filtration using a funnel, a clean cloth and a filter paper, the macerates were concentrated in a rotary evaporator under vacuum, then kept in an oven (50 ° C) for 3 days to give the dry crude concentrate (TA).

Digestion at 50 ° C and 70 ° C

Using a ground-necked flask topped with a condenser, 100 g of powder was added to 700 mL of distilled water. The mixture was placed in a water bath maintained at 50 ° C and 70 ° C respectively for 30 min. This operation was repeated 3 times with the same marc. After filtration using a funnel, a clean cloth and filter paper, the digest were concentrated in a rotary evaporator under vacuum, then stored in an oven (50 ° C) for 3 days to respectively provide the dry crude concentrates TB (digestion at 50 ° C) and TC (digestion at 70 ° C).

Decoction

For 30 min, 100 g of vegetable powder in 700 mL of distilled water was brought to a boil. The operation was repeated 3 times with the same marc. After concentration, the decoctate was dried in an oven (50 ° C) for 3 days to provide the TD aqueous crude extract.

Preparation of selective extracts

One mass of each crude extract (1 g) was dissolved in distilled water (15 mL). The resulting solution was treated successively with hexane, chloroform, ethyl acetate and n-butanol (3 × 5 mL). The different organic fractions were analyzed by TLC.

2.2.2. Phytochemical screening by TLC

The TLC analyzes were carried out on chromatoplates (silica gel 60 F254, rigid aluminum support, Merck). 2 µL of each selective extract from the dry crude concentrates were deposited at reference points (1 cm from each other) on the baseline, drawn 1 cm from the bottom of the plate using capillary, and then allowed to dry before introducing the plate into the developing tank. After revelation of the chromatograms, the colors of the molecular fingerprints were observed and the frontal ratios (Rf) calculated [3-6].

2.2.3. Evaluation of antioxidant potential

Revelation of antioxidant power by TLC

10 µL of each selective extract solution were placed at benchmarks on a chromatoplate, placed in a tank saturated with the developer (migration solvent). The chromatograms obtained were dried and then revealed with an ethanolic

solution of the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH, 2 mg/mL). After 30 min of optimal time, the areas of DPPH trapping activity appear as pale yellow spots on a purple background [7, 8].

Measurement of antioxidant activity by spectrophotometry

The DPPH was solubilized in absolute ethanol to obtain a solution of 0.03 mg/mL. A range of concentrations of each plant sample (0.25 mg/mL; 0.125 mg/mL 0.0625 mg/mL, 0.0313 mg/mL, 0.0156 mg/mL and 0.0078 mg/mL) was prepared with the same solvent. In test tubes containing 1 mL of plant extract of given concentration, 2 mL of the ethanolic solution of DPPH (0.03 mg/mL) were added. The tubes were incubated for 30 min in the dark, and the absorbance is read at 517 nm with a UV-Visible spectrophotometer (AL 800 Spectro Direct). Quercetin served as a positive reference [2, 9]. The percentage reduction of the DPPH of each plant extract to be tested was estimated by the following formula:

$$\% \text{ RP} = [(A_b - A_e) / A_b] \times 100 \quad (1)$$

% RP= Reduction percentage; A_b = absorbance of blank (nm); A_e = absorbance of the sample (nm).

The concentrations necessary to reduce 50% (CR_{50}) of DPPH radical were determined with Excel software.

The median effective concentration (EC_{50}) was calculated using the following equation:

$$EC_{50} \text{ (mg of extract / mg DPPH)} = \frac{CR_{50}}{[DPPH]} \quad (2) \quad [10]$$

2.2.4. Assessment of antibacterial activity

Extract sterility test

The various extracts were seeded on MH agar plates and incubated at 37 ° C. for 24 h. After this period, the extracts to be tested were declared sterile, if no germ is visible on the various agars [11, 12].

Efficiency test

A range of extract concentrations (125 mg/mL, 250 mg/mL and 500 mg/mL) were prepared. The samples obtained were introduced into wells made on the agars previously seeded [12, 13]. Sterile water and the AMC antibiotic (amoxicillin + clavulanic acid) used in the treatment of dental caries served as negative and positive controls, respectively.

Determination of MIC, MBC and MBC/MIC antibacterial parameters

The Minimum Inhibitory Concentrations (MIC) and Bactericidal (MBC) were determined by dilution in liquid medium [12-14]. The MBC/MIC ratio made it possible to specify the modality of action of the tested sample [15].

3. Results and discussion

3.1. Extraction yields

Water has been used as an extraction solvent to comply with the traditional use of *Cocos nucifera* L. in the treatment of dental caries [16]. The different yields of the extractions obtained are listed in Table 1. The yields vary from one extraction technique to another. At high temperatures, the extraction efficiency is significant. Digestion at 70 ° C and decoction provided the best yields (12.82% and 11.58% respectively).

Table 1 Yields of the different extractions

	TA	TB	TC	TD
Powder mass (g)	100	100	100	100
Extract mass (g)	8.05	8.2	12.82	11.58
Yield (%)	8.05	8.2	12.82	11.58

TA: *C. nucifera* extract at room temperature; TB: *C. nucifera* extract at 50°C;
TC: *C. nucifera* extract at 70°C; TD: extract of *C. nucifera* decocted

These results could explain the use of these extraction methods most often used in traditional therapy. Indeed, the moderate increase in temperature seems to activate the extraction of soluble phytochemicals, and therefore allow better optimization of the extractions. The drop in yield observed for the TD extract could be explained by the degradation of certain thermosensitive phytochemicals at 100 °C (water boiling point). The TA and TB extraction yields are insignificant. This seems to be justified by too short an extraction time, which would limit optimal turgor of the cells in order to release the extractable phytochemicals by bursting.

3.2. Phytochemical composition

3.2.1. Hexane extracts

The Lieberman-Burchard reagent revealed in UV light / 366 nm, triterpenes and steroids while the alkaline solution of potassium hydroxide (5% KOH in methanol) demonstrated the presence of coumarins [6, 8]. The chromatographic profiles observed differ from one extract to another (Table 2). Indeed, the sterols observed (spots of Rf = 0.21 and Rf = 0.51) present in TA are absent in the 3 other extracts. This could therefore be justified by the rise in temperature which must have degraded these compounds. KOH detected a coumarin (Rf = 0.19) under UV light / 366 nm in the TB, TC and TD extracts, which is absent in the TA extract. The presence of this compound would be due to the temperature which is favorable for its extraction. Also, the results show that the more the temperature increases, the less sterols there are and the more coumarins there are in the extracts.

Table 2 Phytochemicals detected in the different hexane extracts.

Extract	Rf	
	Lieberman-Burchard	KOH
	Steroids	Coumarins
TA	0.21 ; 0.31 ; 0.51 ; 0.57	0.30 ; 0.44 ; 0.75
TB	0.29 ; 0.50	0.19 ; 0.31 ; 0.74 ; 0.79
TC	0.29 ; 0.55 ; 0.71	0.19 ; 0.31 ; 0.74 ; 0.79
TD	0.52	0.19 ; 0.31 ; 0.74 ; 0.79 ; 0.81

TA: *C. nucifera* extract at room temperature; TB: *C. nucifera* extract at 50°C;
TC: *C. nucifera* extract at 70°C; TD: extract of *C. nucifera* decocted

3.2.2. Chloroform extracts

AlCl₃ (1% in ethanol), Godin, KOH and Liebermann reagents [17, 18] were used to detect the presence of coumarins, flavonoids, steroids and triterpenes in the extracts tested (Table 3).

The molecular fingerprints in the form of spots testifying to the presence of these different compounds were observed in the TD extract, then in the TC and TB extracts than in the TA extract.

Table 3 Phytochemicals detected in the various chloroform extracts.

Extract	Rf		
	Lieberman-Burchard	KOH	AlCl ₃ ; Godin
	Triterpenes	Coumarins	Flavonoids
TA	0.14	0.04 ; 0.09 ; 0.14 ; 0.20 ; 0.27 ; 0.31 ; 0.56	0.11 ; 0.51
TB	0.07 ; 0.15 ; 0.26 ; 0.62	0.05 ; 0.09 ; 0.12 ; 0.29 ; 0.35 ; 0.44 ; 0.54 ; 0.79 ; 0.89	0.11 ; 0.45
TC	0.15 ; 0.24	0.05 ; 0.07 ; 0.16 ; 0.19 ; 0.25 ; 0.39 ; 0.47 ; 0.56 ; 0.80 ; 0.90	0.20 ; 0.42 ; 0.44 ; 0.49 ; 0.80
TD	0.15 ; 0.21 ; 0.29 ; 0.64	0.04 ; 0.07 ; 0.11 ; 0.16 ; 0.27 ; 0.31 ; 0.46 ; 0.50 ; 0.82 ; 0.89	0.35 ; 0.37 ; 0.50

TA: *C. nucifera* extract at room temperature; TB: *C. nucifera* extract at 50°C;
TC: *C. nucifera* extract at 70°C; TD: extract of *C. nucifera* decocted

3.2.3. Ethyl acetate extracts

The phytoconstituents identified by TLC in the extracts (TA, TB, TC, and TD) after revelation with KOH, AlCl₃, Godin's reagent, and FeCl₃ are flavonoids, coumarins and tannins (Table 4). The chromatographic profiles of the samples are almost identical. In view of the number of molecular spots observed, the various extracts are mainly composed of phenolic compounds.

Table 4 Phytocompounds detected in the various ethyl acetate extracts

Extract	Rf		
	KOH	AlCl ₃ ; Godin	FeCl ₃
	Coumarins	Flavonoids	Tannins
TA	0.27 ; 0.50 ; 0.55; 0.61; 0.70	0.15 ; 0.19; 0.42; 0.67; 0.75; 0.70; 0.87	0.51
TB	0.27 ; 0.50 ; 0.55; 0.61; 0.70	0.12 ; 0.19; 0.42; 0.67; 0.75; 0.77; 0.87	0.51
TC	0.27 ; 0.40; 0.46; 0.54; 0.70; 0.81	0.12 ; 0.19; 0.42; 0.67 ; 0.75 ; 0.77	0.51
TD	0.27; 0.46; 0.50; 0.55; 0.61; 0.70 ; 0.81	0.12, 0.20; 0.42; 0.67; 0.77	0.50

TA: *C. nucifera* extract at room temperature; TB: *C. nucifera* extract at 50°C;
TC: *C. nucifera* extract at 70°C; TD: extract of *C. nucifera* decocted

3.2.4. n-Butanol extracts

The groups of compounds identified (Table 5) are: coumarins, flavonoids and tannins.

Table 5 Phytocompounds detected in the different n-butanol extracts

Extract	Rf		
	KOH	AlCl ₃ ; Godin	FeCl ₃
	Coumarins	Flavonoids	Tannins
TA	0.66	0.32 ; 0.46; 0.70	0.25 ; 0.62
TB	0.56 ; 0.66 ; 0.74	0.32 ; 0.46 ; 0.71	0.25 ; 0.62 ; 0.75
TC	0.56 ; 0.66 ; 0.74	0.32 ; 0.46 ; 0.71	0.25 ; 0.62 ; 0.75
TD	0.56 ; 0.66; 0.74	0.32, 0.46 ; 0.71	0.25 ; 0.62 ; 0.75

TA: *C. nucifera* extract at room temperature; TB: *C. nucifera* extract at 50°C;
TC: *C. nucifera* extract at 70°C; TD: extract of *C. nucifera* decocted

The study of n-butanol extracts showed virtually the same compounds (coumarins, flavonoids and tannins) in the 4 extracts revealed with KOH, AlCl₃, Godin's reagent, and FeCl₃. However, we still see the appearance and disappearance of certain compounds from one extract to another. Compounds such as coumarin (Rf = 0.74); flavonoid (Rf = 0.71) and tannin (Rf = 0.75) absent in the TA extract, are observed in the TB, TC, and TD extracts.

3.3. Antioxidant activity

3.3.1. Antioxidant profile by TLC

Table 6 shows the antioxidant profile of the different extracts. The appearance of the pale yellow spot on a purple background, visible to the naked eye, attests to the presence of anti-radical compounds [7]. TLC was performed under the same conditions as the phytochemical screening. After development, it appears that the TB and TC digests contain more anti-free radical compounds, but much more in TC.

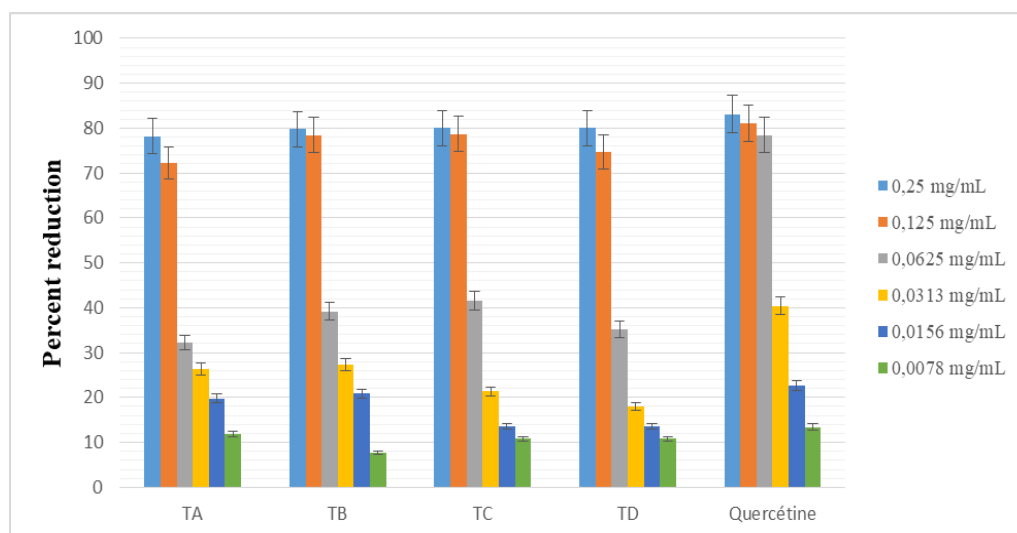
Table 6 Antioxidant phytochemicals observed in the different extracts analyzed.

Extract	Rf		
	Triterpenes	Coumarins	Flavonoids
TA		0.09 ; 0.42 ; 0.56	0.15 ; 0.42 ; 0.61 ; 0.81
TB	0.15 ;	0.09 ; 0.42 ; 0.56 ; 0.61 ; 0.79	0.12 ; 0.42 ; 0.71 ; 0.81
TC	0.15	0.07 ; 0.39 ; 0.56 ; 0.61 ; 0.79 ; 0.90	0.12 ; 0.42 ; 0.71 ; 0.81
TD	0.15 ; 0.21	0.56 ; 0.61 ; 0.90	0.12 ; 0.42 ; 0.71 ; 0.81

TA: *C. nucifera* extract at room temperature; TB: *C. nucifera* extract at 50°C;
TC: *C. nucifera* extract at 70°C; TD: extract of *C. nucifera* decocted

3.3.2. Antioxidant profile by spectrophotometry

The antioxidant activity of the crude extracts was evaluated spectrophotometrically using the DPPH against quercetin, which is a natural antioxidant taken as a reference. The results obtained (Figure 1) show the percentages of reduction of extracts and of quercetin. Quercetin showed the best antioxidant profile compared to the extracts analyzed starting from the highest concentration (0.25 mg/mL) to the lowest concentration (7.8 µg/mL). In contrast, all 4 extracts exhibited almost similar antioxidant activity at 0.25 and 0.125 mg/mL. These results reflect those obtained by CCM. The concentration of the sample which reduces 50% of DPPH (CR₅₀) and the half effective concentration (EC₅₀) which is an index of effectiveness were determined in mg of antioxidant per mg of DPPH (Table 7).

**Figure 1** Antioxidant Profile of TA, TB, TC, TD and Quercetin Extracts

TA: *C. nucifera* extract at room temperature; TB: *C. nucifera* extract at 50°C;
TC: *C. nucifera* extract at 70°C; TD: extract of *C. nucifera* decocted

Table 7 Synoptic of CR₅₀ and CE₅₀

	TA	TB	TC	TD	Quercetin
CR ₅₀	0.09	0.0798	0.0767	0.0859	0.0391
EC ₅₀	3.00	2.659	2.558	2.865	1.303

The consolidated results (Table 7), reveal that the digests (TB and TC) present a more significant antioxidant profile than the TA and TD extracts because the values of CR₅₀ and EC₅₀ are the lowest. Indeed, the lower the value of the EC₅₀, the more the antioxidant activity of the sample is significant [19].

3.4. Anticariogenic profile

3.4.1. Sterility of extracts

The sterility test was performed on the extracts to verify the absence of germ. No germs were observed in the Petri dishes after their incubation. The TA, TB, TC and TD extracts tested are therefore sterile.

3.4.2. Diffusion in a solid medium of extracts and antibiotic

Table 8 shows the sensitivity of bacteria to extracts TA, TB, TC and TD, at 500 mg/mL and the standard antibiotic used in the treatment of dental caries. The comparison is made between the extracts with the negative control which is sterile distilled water which has shown no efficacy against bacteria. Consequently, the effects observed are mainly due to the phytochemicals contained in the samples [20].

The results obtained from the extracts against the bacterial strains of *S. mutans* tested gave the diameter averages of the zones of inhibition of between 9.33 and 13.33 mm. Against the bacterial strain *S. mutans* 1143C/14, the crude plant extracts exhibited the largest diameters of zones of inhibition compared to the antibiotic tested.

Table 8 Diameter (mm) of the inhibition zones of the samples at 500 mg/mL.

Strains	AMC	TA	TB	TC	TD
<i>S. mutans</i> 1142C/14	34±0.00	9.33±0.44	9±0.00	9±0.00	11±0.67
<i>S. mutans</i> 1143C/14	8±0.00	10±0.67	10±0.00	10±0.00	13.33±0.44

The diameters of the zones of inhibition (Table 8) suggest that the bacterial strains are sensitive to the extracts tested. Indeed, a bacterium is said to be resistant to an extract when the diameter of the zone of its inhibition is less than or equal to 8 mm, sensitive if this diameter is between 9 and 14 mm, very sensitive when it is between 15 and 19 mm and extremely sensitive for a diameter greater than or equal to 20 mm [21].

3.4.3. MIC and MBC antibacterial parameters

After 24 h incubation at 37°C under CO₂ (5%) of the germs, a progressive decrease in bacterial growth in the microplate wells according to the different concentrations of the extracts tested compared to the growth control was observed. Tests to determine the antibacterial parameters MIC and MBC were carried out in order to specify the nature of the antibacterial activity of the extracts.

Table 9 Antibacterial parameters of the extracts tested

Strain	Extract	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Interpretation
<i>Streptococcus mutans</i> 1142C/14	TA	62.5	62.5	1	Bactericidal
	TB	125	125	1	Bactericidal
	TC	62.5	62.5	1	Bactericidal
	TD	31.25	31.25	1	Bactericidal
<i>Streptococcus mutans</i> 1143C/14	TA	125	125	1	Bactericidal
	TB	62.5	62.5	1	Bactericidal
	TC	125	125	1	Bactericidal
	TD	62.5	62.5	1	Bactericidal

The MIC and MBC values determined with respect to the bacterial strains have been collated in Table 9. The MBC/MIC report made it possible to specify the modality of action of the extracts according to the method described by Fauchere (2002) [15]. Indeed, an extract is said to be bactericidal if the MIC/MBC ratio is less than or equal to 2.

The TA, TB, TC and TD extracts are bactericidal vis-à-vis the bacterial strains tested. The observed anticariogenic activity is linked to the co-presence of triterpenes, flavonoids and tannins [22]. The extracts all exhibited bactericidal power, indicating that temperature would have no effect on the observed biological activities.

4. Conclusion

The main objective of the present study was to study the influence of temperature on the phytochemical composition and the antioxidant and anticariogenic activities of the husk of the fruit of *Cocos nucifera* L. (Arecaceae). Phytochemical analysis by TLC revealed the coexistence of coumarins, flavonoids, steroids, tannins, and triterpenes from the husk of the yellow fruit of the plant studied. However, the chromatographic profiles showing the phytochemical composition of the extracts vary somewhat as a function of the temperature. The antioxidant potency study with DPPH showed a moderate antioxidant profile overall for extracts from the fruit husk of *C. nucifera* compared to quercetin. The bioassay revealed the bactericidal effect of TA, TB, TC and TD extracts against strains of *Streptococcus*. These results show prospects for the use of the fruit shell in dental care.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

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