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Assessment of the antioxidant capacity and total polyphenol and flavonoid contents of extracts from three plants used in the treatment of menopausal symptoms in Côte D'ivoire

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Abstract

Polyphenols and flavonoids, two major classes of phytochemicals, are known for their antioxidant, anti-inflammatory and hormonal properties. Their presence in plants may help alleviate menopausal symptoms, such as hot flashes, irritability and sleep disturbances, by neutralizing free radicals and modulating inflammatory responses. The aim of this study was to assess the total polyphenol and flavonoid contents of three plants, and to investigate their antioxidant potential. The spectrophotometric determination of total polyphenols and flavonoids and the quantitative evaluation of the antioxidant activity towards the stable radical 1,1- diphenyl-2-picrylhydrazyl (DPPH) of three plants used in the treatment of menopausal symptoms in Côte d'Ivoire were carried out. Results showed that methanolic extracts of the leaves of *Manihot esculenta*, *Talinum fruticosum* and *Khaya senegalensis* contained varying levels of both polyphenols and total flavonoids. The methanolic extracts of the leaves of *M. esculenta*, *K. senegalensis* and *T. fruticosum*, revealed that the leaves of *M. esculenta* and *K. senegalensis* respectively contain the highest quantities of polyphenols (55896.55 µg EAG/g DM; 35810.35 µg EAG/g DM) and flavonoids (1943.66 ± 65.18 mg EQ/g DM; 1780.92 ± 173.27 mg EQ/g DM). For antioxidant activity, the IC_{50} values observed showed that *M. esculenta* (IC_{50} = 0.122 ± 0.0005 mg/ml) and *K. senegalensis* (IC_{50} = 0.227 ± 0.0015 mg/ml) extracts were the most active after ascorbic acid used as a control. Regular consumption of these plants could play an important role in controlling menopausal symptoms.

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Introduction

Plant uses by human merges with humanity history. They are used for nutritional purposes, against diseases and certain physiological changes observed with the ageing in human such as menopause (Macheix *et al.*, 2005; Amiot, 2023). Menopause is a physiological state in women's lives, which is a definitive interruption of their ovulation and menstrual function (WHO 2022). It is characterized by several symptoms such as hot flashes, sleep disturbances, mood swings, joint and muscle pain (Clere, 2017), cardiovascular diseases, osteoporosis and other physiological manifestations (Thurston *et al.*, 2021). Thus, medicinal plants are used to relieve menopausal symptoms due to their accessibility, affordability and therapeutic potential (Agban *et al.*, 2013). In Côte d'Ivoire, surveys carried out among women of menopausal age made it possible to identify plants used in the treatment of menopausal symptoms including *Manihot esculenta*, *Talinum fruticosum* and *Khaya senegalensis* (Kouamé *et al.*, 2018). These plants are also used in traditional medicine to treat several health conditions similar to the symptoms of menopause (Rao *et al.*, 2013; Laleye *et al.*, 2015; Etame-Loe *et al.*, 2018).

Found inside many plants, polyphenols and flavonoids are natural compounds of plants that have demonstrated beneficial properties in human health (Macheix *et al.*, 2005). These are phytochemicals, known for their antioxidant and anti-inflammatory properties (Schnarr *et al.*, 2022). Polyphenols like isoflavones are phytoestrogens able to reduce disorders linked to menopause (Cheng *et al.*, 2007; Chen *et al.*, 2015; Delia *et al.*, 2017). These phytonutrients appear essential to the accurate functioning of the body and help protect it against certain disorders linked to menopause such as cardiovascular diseases, cancers and degenerative diseases (Macheix *et al.*, 2005; Steinshamn *et al.*, 2008). The presence in plants can thus help to alleviate the symptoms of menopause by neutralizing free radicals and modulating inflammatory responses. This study is devoted to three plants used in the treatment of menopausal symptoms in Côte d'Ivoire. Its aim is to evaluate polyphenols and flavonoids total contents in the plants and to measure free radicals scavenging activity.

Materials and methods

Plant material

Plant material is essentially made up of leaves of three collected following an ethnobotanical survey. These are *Manihot esculenta* Crantz, *Talinum fruticosum* (L.) Juss. and *Khaya senegalensis* (Desr.) A. Juss. These plants were selected following a qualitative and quantitative study of phytoestrogens in 27 plants used by women of menopausal age in Côte d'Ivoire (Kouamé *et al.*, 2022). Quantity of isoflavones, the presence of lignans in the different plants are the criteria which motivated the choice of plants.

Preparing the extracts

The preparation of the crude extracts was done according to the modified method described by Isabela *et al.* (2008). For this purpose, 10 g of plant material were macerated in 80 mL of methanol for 24 h with mechanical stirring. After that, the volume of the solution was adjusted to 100 mL with methanol and the mixture was filtered with Whatman No. 4 paper and cotton. Part of the filtrate obtained was used for the qualitative analysis of isoflavones and the other part was concentrated in an oven for one week at 40°C for the quantitative analysis of these compounds.

Determination of total polyphenols

The total phenolic content was determinated according to the Folin-Ciocalteu colometric method (Heilerova *et al.*, 2003). The sample (1ml) and 1.5 ml of sodium carbonate (17 %, w/v) were added to 0.5 ml of Folin-Ciocalteu reagent (0.5 N). After 30 min of reaction at room temperature, the absorbance was measure at 760 nm. The total phenol content was calculated from gallic acid calibration curve (0- 35 µg / ml) and expressed in µg of gallic acid equivalent/g of dry matter (µg EAG/g DM).

The total polyphenol content (Q) was calculated according to the following formula:

$$Q\ (\mu g\ EAG/g) = (V \times C \times F_d) / m\ (\mu g\ AG/g)\ dry\ matter \quad (1)$$

V: filtrate volume

C: extract concentration

Fd: dilution factor

m: mass of dry matter

Determination of total flavonoids

The total flavonoid content 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 total flavonoids (F) is calculated in quercetol equivalent according to the following formula 2.

$F = (0.05 \times A_{ext} / A_q) \times F_d \times 100) / C_{ext}$ (en %) (2)

Aext : Absorption of extract

Aq : Absorption of quercetol

Cext : Extract concentration

Fd : Dilution factor

The proportions of total flavonoids contents determined were subsequently expressed in mg EAG/ g DM relative to the total phenolic.

The quantity of total flavonoids (QF) of total flavonoids was calculated using the polyphenol content and the total flavonoids contents the percentage of flavonoids using the following formula:

QF (mg EQ/g dry matter) = Q × F (3)

Q: total polyphenol content

F: percentage of total flavonoids

The quantity of flavonoids in the different extracts was expressed in mg of quercetol equivalent per g of extract (mg EQ/g).

Assessmentof antioxidant activity

The antioxidant activity was analyzed using the stable radical 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay with slight modifications (Blois 1958). Moreover, the DPPH radical-scavenging activity was determined on the different extracts. 1 ml of various concentrations of the methanolic samples (1 mg/ml; 0.5 mg/mL; 0.25 mg/mL; 0.125 mg/mL; 0.0625 mg/mL; 0.0313 mg/mL; 0.0156 mg/mL; 0.0078 mg/mL; 0.0039 mg/mL; 0.002 mg/mL) were added to 2 ml of 0.03 mg/ml ethanolic solution of DPPH. After an incubation of 30 min of incubation the absorbance was read at 517 nm against a blank (1 ml ethanol and 2 ml DPPH solution) with UV-visible spectrophotometer.

The positive reference control is ascorbic acid, commonly known as vitamin C. The percentages of DPPH inhibition was calculated according to the formula:

$PI = [1-(Ae/Ab)] \times 100$ (4)

PI: percentage of DPPH inhibition

Ae: sample absorbance

Ab: absorbance of white

Concentrations necessary to trap 50% of the DPPH (IC_{50}) were determined with Excel software from a calibration curve with the equation y = ax + b obtained using the different concentrations of vitamin C.

The IC_{50} value is the extract concentration that inhibits 50% of DPPH. Thus, the lower value of the inhibition concentration means greater antioxidant activity. Moreover, the graph drawn the link between the inhibition of concentration and the concentration of each sample of vitamin C made it possible to calculate the IC50 using the following formula:

IC_{50} (mg/mL) = $[(I_{50}-b)/a]$ (5)

I_{50} : inhibition percentage corresponding to 50%

a and b : curve coefficients

Statistical analyzes

The average of different quantities of compounds were calculated with Excel software and were subjected to a one-way analysis of variance (ANOVA) using the Graph Pad Prism 5 software. When a difference is observed for each character ($p < 0.05$), the variance is completed by comparing the means using Tukey's multiple comparison test at the threshold of 0.05. The correlation between the antioxidant activities of the extracts and their phytocompound contents was established by the R^2 value obtained through the construction of a point cloud. If $R^2 < 0.10$, there is no correlation; $R^2 < 0.50$, the correlation is weak. If $0.50 \geq R^2 < 0.80$, the correlation is intermediate and $R^2 \geq 0.80$, the correlation is strong (Prabhjit *et al.*, 2008; Kouassi, 2018).

Results

Total polyphenols contents

The total polyphenol content was obtained from the linear regression equation of gallic acid (y= 0.0232 x + 0.0002) (Fig. 1). The contents of the methanolic extracts of the leaves of *K. senegalensis*, *M. esculenta* and *T. fruticosum* ranged from 35810.35 to 55896.55 µg EAG/g DM (Fig. 2). The leaf extract of *M. esculenta* (55896.55 µg EAG/g DM) contains the highest content, followed by *K. senegalensis* (53267.24 µg EAG/g DM) and *T. fruticosum* (35810.35 µg EAG/g DM). The results showed that there is a highly significant difference between the values (α = 0.05 and p < 0.0001). However, there was no significant difference between the total polyphenol contents of *M. esculenta* and *K. senegalensis* extracts.

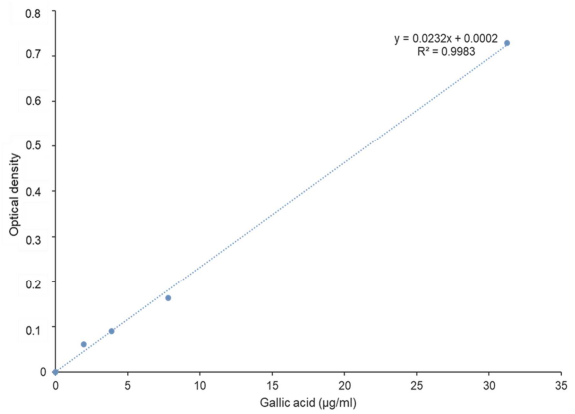


Fig. 1. Gallic acid calibration line for the determination of total polyphenols

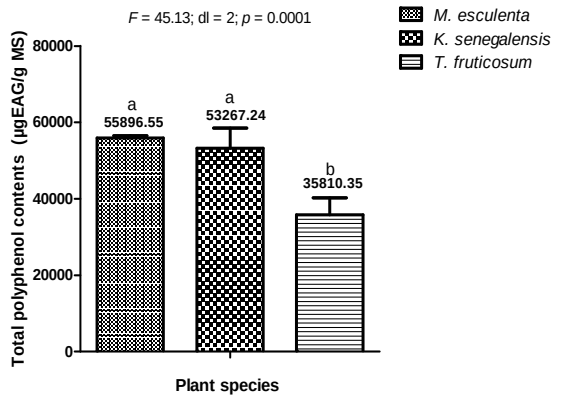


Fig. 2. Total polyphenol contents of methanolic extracts of *M. esculenta*, *K. senegalensis* and *T. fruticosum*

Total flavonoids contents

The percentage of total flavonoid content was calculated from total polyphenol content and expressed as µg EAG/g DM. The total flavonoid contents determined in the plant extracts are shown in Table 1. The results indicated that the proportions of total flavonoids range from 529.72 to 1943.66 mg EQ/g DM. *M. esculenta* had the highest total flavonoid content at 1943.66 mg EQ/g DM. *T. fruticosum* had the lowest content at 529.72 mg EQ/g DM. The ANOVA 1 test showed that there was a highly significant difference between the means of the different extracts (α = 0.05 and p < 0.0001). However, there was no significant difference between the extracts of *M. esculenta* and *K. senegalensis*.

Table 1. Total flavonoid contents of methanolic extracts of *M. esculenta*, *K. senegalensis* and *T. fruticosum*

Plant species	Organs used	F (%)	Content±SD mg EQ/g
Manihot esculenta	Leaves	34.77	1943.66±65.18[a]
Khaya senegalensis	Leaves	33.43	1780.92±173.27[a]
Talinum fruticosum	Leaves	14.79	529.72±71.21[b]
dl			2
F			273.70
p			<0.0001

F (%) = percentage of total flavonoid

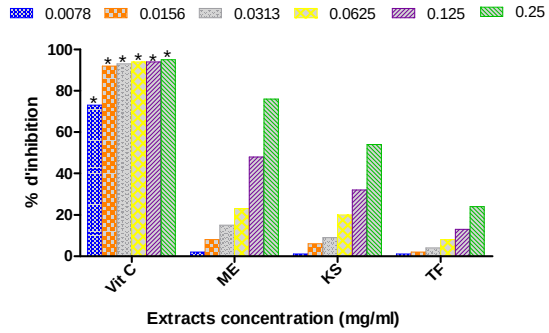
Values with the same letter do not show a significant difference (The difference is significant for p< 0.0001 with the ANOVA test)

Antioxidant activity

Inhibition percentage

The concentration-dependent inhibition percentages obtained for each plant extract and for the standard are shown in Fig. 3. The DPPH inhibition percentages for these extracts ranged from 1.82% to 75.68% for the *M. esculenta* extract, from 1.13% to 53.64% for the *K. senegalensis* extract and from 0.98% to 24.40% for the *T. fruticosum* extract. The inhibition percentages for the standard (vitamin C) ranged from 73.07% to 94.77%. Vitamin C and *M. esculenta* extract therefore showed the best antioxidant activity. The one-way analysis of variance showed a highly significant

difference at the α threshold of 5% with $p < 0.001$ between the different percentages of inhibition of ascorbic acid and that of the extracts of the species tested.



Vit C : Vitamin C; ME : M. esculenta; KS : K. senegalensis; TF : T. fruticosum

Fig. 3. Percentages of DPPH inhibition of the methanolic extracts tested

Inhibition concentration (IC_{50})

The IC_{50} values determined are given in Table 2. *M. esculenta* extract had the lowest IC_{50} value. Thusly *M. esculenta* extract (0.122 mg/ml) exhibited better antioxidant activity compared to *K. senegalensis* (0.227 mg/ml) and *T. fruticosum* (0.583 mg/ml) extracts. However, it was less active than vitamin C (0.0049 mg/ml). The results showed that there is a highly significant difference between these values at the α threshold of 0.05 with $p < 0.0001$.

Table 2. Inhibition concentrations 50 (IC_{50}) of methanolic extracts of the plants studied

Standard and plant species	Organs used	IC_{50} (mg/ml)±SD
Ascorbic acid		$0.0049±0.0183^{a}$
Manihot esculenta	Leaves	$0.122±0.0005^{b}$
Khaya senegalensis	Leaves	$0.227±0.0015^{c}$
Talinum fruticosum	Leaves	$0.583±0.0009^{d}$
dl		3
p		<0.0001

Values with the same letter do not show a significant difference (The difference is significant for $p < 0.0001$ with the ANOVA test)

Correlation between antioxidant activity and phytocompounds

The correlation coefficients (Fig. 4 and 5) established between the phytocompound content of plant extracts and antioxidant activity was highly significant (R^2 = 0.99 for polyphenols and R^2 = 0.98 for flavonoids). The coefficient R^2 = 0.99 indicated that 99% of the capacity of the extracts is due to the contribution of the phenolic compounds and R^2 = 0.98 indicated that 98% of the antioxidant potential is due to the presence of the flavonoids contained in the plant extracts.

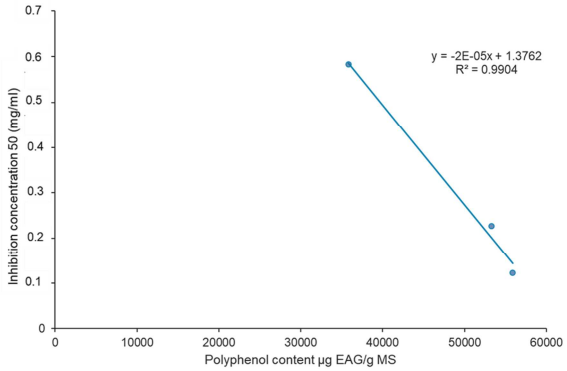


Fig. 4. Correlation curve between the inhibition concentration 50% of the DPPH radical and the polyphenol content

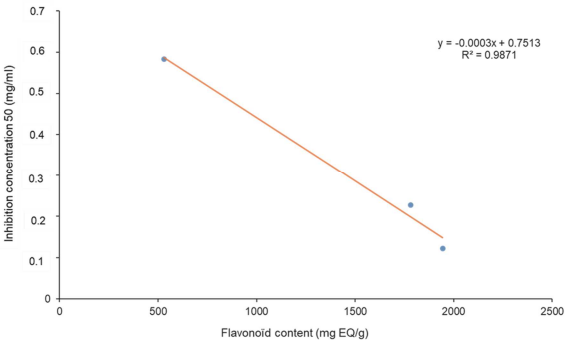


Fig. 5. Correlation curve between the inhibition concentration 50% of the DPPH radical and the flavonoid content

Discussion

This study was carried out to determine the antioxidant activity, total phenol and flavonoid contents of extracts from three plants used in the treatment of menopausal symptoms in Côte d'Ivoire. The total polyphenol contents of the plant studied extracts ranged from 35810.35 to 55896.55 µg EAG/g MD. Furthermore, the total flavonoid content of the extracts varied from 529.72 to 1943.66 µg EAG/g DM. The *M. esculenta* leaf extract showed the highest

polyphenol and total flavonoid contents. The presence of polyphenols and flavonoids in these plants could explain their use in traditional medicine.

This result suggests that the anti-free radical activity of these plants is due to the presence of polyphenols and flavonoids compounds. The correlation between antiradical activity and polyphenol and flavonoid content has previously been established in several researches (N'guessan *et al.*, 2007; Koné 2009; Moussa *et al.*, 2018). According to Hsu *et al.* (2007), phenolic compounds are widely distributed in plant tissues, including many antioxidant molecules. In addition, several studies have shown a correlation between total phenol content and antiradical activity (Duh *et al.*, 1999; N'guessan *et al.*, 2007; Koné, 2009). According to Chen and Ho (1995), the functional groups present in phenolic compounds in general can easily give up an electron or proton to neutralize free radicals. The high antioxidant activity of these plants is therefore linked to their high phenolic compound content. These plants contain flavonoids with good antioxidant activity (N'guessan *et al.*, 2007). The antioxidant activity and polyphenol and flavonoid contents of these plants have already been reported. Several studies have shown that *K. senegalensis* (Mane, 2012; Monon *et al.*, 2019), *T. fruticosum* (Liao *et al.*, 2015; Moussa *et al.*, 2018) and mild varieties of *M. esculenta*, including variety 9620A (Brou *et al.*, 2010; Faezah *et al.*, 2013) contained varying amount of polyphenols and flavonoids with remarkable antioxidant activities. The results of this study therefore confirm these previously reported findings.

Polyphenol compounds have a pronounced antioxidant activity (Hodek *et al.*, 2002; Yue *et al.*, 2010) and are therefore able to fight against skin ageing (Sacks *et al.*, 2006), one of the symptoms of the menopause. Flavonoids inhibit LDL oxidation and, thanks to their antioxidant activity, can prevent atherosclerosis and reduce the risk of cardiovascular disease. Also due to their anti-tumor and anti-cancer potential (Yang *et al.*, 2001), these compounds could provide relief for post-menopausal women at risk of cancer. The phytochemical richness of *M. esculenta*, *K. senegalensis* and *T. fruticosum* is a field of investigation with a view to developing alternative therapies for the management of menopausal symptoms.

Conclusion

The determination of polyphenols and flavonoids, is carried out on the methanolic extracts of the leaves of *Manihot esculenta*, *Khaya senegalensis* and *Talinum fruticosum*, revealed that the leaves of *Manihot esculenta* and *Khaya senegalensis* contain respectively the highest quantities of polyphenol.

The study is shown that leaves of *M. esculenta* and *K. senegalensis* respectively possess the strongest anti-radical activities. For these plants, there is a correlation (R_2 > 0.50) between the anti-radical activity and the contents of polyphenols, flavonoids and isoflavones. Regular consumption of these plants could manage menopausal symptoms, but this indication must be validated by further investigations on an oestrogenic activity.

Recommendation(s)

Extracts from the three plants studied showed high levels of polyphenols and flavonoids. It would therefore be interesting to evaluate the estrogenic activity of these plants with a view to better management of menopausal symptoms.

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