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RESEARCH ARTICLE

EFFECT OF MACERATION DURATION ON THE YIELD, QUALITY OF BIOMOLECULES AND ANTI-RADICAL POWER OF PERICARPUS EXTRACTS OF MANGOSTAN FRUITS GARCINIA GAERTN ACCLIMATED IN CONGO-BRAZZAVILLE

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ABSTRACT

The aim of this study is to evaluate the effect of maceration time on the yield, quality of biomolecules and anti-radical power of pericarp extracts of *Mangosteen Garcinia fruits*. Phytochemical detection was carried out from ethanolic extracts obtained at different consecutive times, 24, 48 and 72 hours of maceration with 50g of powder of dried pericarps of mamgoustan fruits, thin layer chromatography followed by fluorescence detection, in vitro tests for evaluating the anti-radical power visualized with a UV-visible spectrophotometer were observed. The proximal composition by qualitative dosage of total polyphenols was carried out by the universal methods described by Folin-ciocalteu and flavonoids by the $AlCl_{3\ method}$ through the sight of the UV- visible spectrophotometer. The anti-radical power was measured by the DPPH test. The much higher dry matter contents appear from 72 hours, extraction times of E3, or 7.08% in extraction yield. The compounds identified include tannins, flavanols, leuco anthocyanins, alkaloids and saponins, with a strong predominance of polyphenols. Samples E1, E2 and E3 contain respectively the total polyphenol contents of the order of 2033.06 mgEAG / g Ms, 2188.86 mgEAG / gMs and 1796.86 mgEAG / gMs and with the same trends as the total flavonoid contents of 1234.81 mgEQt / gMs , 1241.42 mgEQt / gMs and 977.17 mgEQt / gMs . The IC50 values are 4.15µg/ml, 4.22µg/ml and 3.70µg/ml for the three samples respectively. The extraction duration has a significant impact on the yield, total polyphenol content, total flavonoids and inhibition potential. E3 has a high inhibition capacity with the lowest IC50 compared to E1 and E2. Mangosteen fruit pericarp extracts contain interesting biactive compounds, thus opening the way for the valorization of these wastes in food and medicine.

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INTRODUCTION

Many of the human body's survival problems are partly solved by eating a diet rich in fruit. Indeed, studies carried out on several fruits show that they possess biomolecules with interesting antioxidant powers (Gouolally, 2020; Hosakatte, 2020). These biomolecules have structures that are the basis for the development of food additives, functional foods and nutraceuticals (Dall'armellina, 2021; Ibrahim *et al*. 2012). In recent years, they have been sources of research in the agri-food, phytotherapeutic, physiological and nutritional fields (Udani *et al*, 2009; Aizat *et al*, 2019) and have a protective effect against major chronic pathologies such as cardiovascular, neurodegenerative diseases, diabetes, cancers and have an acceptable energy content, thus preventing the rapid development of overweight (ReddyA, 2016; Johnson *et al*, 2021; Abuzaid *et al*., 2016). Apart from the pulp which is the edible part of the fruit, the pericarps are often considered waste for consumers even though they contain bioactive compounds important for health (Gorinstein *et al*., 2002); Guyot *et al*. 2002). The same is true for the pericarps of *Mangosteen Garcinia Gaertn*which have been used for decades in traditional medicine to treat multiple diseases due to its high content of phytocompounds identified as xanthones (Rynyah *et al*, 2022; Mario Abate *et al*, 2020; Sukaka *et al*. 2013; Wittenauer *et al*., 2012).

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However, assessing biological activities requires mastery of the extraction method of these biactive compounds. In the food industry, the most widely used method is maceration and ethanol is the best solvent for extracting sensitive biomolecules. Maceration is a physicochemical process during which non-volatile biomolecules, including polyphenols, are extracted, in particular anthocyanins, tannins, among other substances such as aromatic compounds, nitrogen compounds, polysaccharides, minerals, etc. (Fang, 2011). The pericarp of the fruit of the Congo-Brazzaville mangosteen cultivar represents two-thirds of its total mass. Most of the time, this quantity of plant material is thrown away by consumers who are unaware of its use as a real source of income for the population. It is in this context that this work is undertaken, the aim of which is to identify the chemical families available inthe extractsof *Mangosteen Garcinia Gaertn* fruit pericarpsacclimatized in Congo- Bazzavile, to determine the contents of total phenolic and flavonoid compounds and to evaluate the anti-radical activity of the ethanolic extracts taking into account the extraction time by the maceration method.

MATERIALS AND METHODS

Plant material studied: *Mangosteen garcinia* which is the subject of this study is a species of flowering plant of the genus Garcinia and belonging to the family of clusiaceae. The fruits come from the orchards of the south of the Republic of Congoin the locality of

Bocko (Pool) (4°13'44" South and 15°7'52" East). After picking, the fruits were sorted, washed in water, weighed and peeled to separate the pericarps and the pulp.

Figure 1. ***Mangosteen garcinia Gaertn*** **fruits (photograph taken by Koumba- moukouama , 2023)**

Preparation of ethanolic extracts of mangosteen fruit pericarps: To perform the assay of polyphenols, total flavonoids and anti-radical activities, the pericarps were dried in a Memmert brand UN30 natural convection oven in the laboratory at 60°C for 24 hours, then ground using a manual mechanical grinder. 50 g of pericarp powder were macerated in 150 ml of 90°C ethanol for 24, 48 and 72 hours respectively. The extracts E1 and E2 and E3 thus obtained in relation to the extraction times were concentrated to dryness using a rotary evaporator and then stored in a cool place (+4°C) for possible analyses. The yields of the different extracts were calculated.

Detection of chemical families by colorimetric methods:The chemical family detection tests were carried out according to the colorimetric methods already described by some researchers (N' guessan et al., 2009).

Detection of chemical families by thin layer chromatography: Thin Layer Chromatography was performed with the extracts. The eluent system and the developer were used for identification of specific chemical groups. This is the toluene /formic acid system (5/5) followed by spraying with Neu's reagent for the detection of polyphenols and flavonoids in particular. They are obtained from a 1% (m/v) solution of 2-aminoethyldiphenylborate and with 5% (m/v) of PEG400 in methanol. Fluorescence is observed in UV light at 365 nm.

Total polyphenols contained in the three pericarp extracts: The quantitative determination of polyphenols was carried out by the method described by Muanda et al. in 2011. In an Eppendorff tube, 0.1 ml of the extract (2 mg/ mL), 0.9 mL of distilled water and 0.9 mL of Folin-Ciocalteu reagent (1N) were mixed. 0.2 ml of a Na2CO3 solution (20 %) was immediately added. The solution thus obtained is incubated at room temperature for 40 min away from light. The absorbance is then measured with a spectrophotometer at 725 nm against an ethanol solution used as a control. It should be noted that the calibration curve was previously established with gallic acid under the same conditions as the extracts to be analyzed.

Determination of total flavonoids contained in the three pericarp extracts: Total flavonoids were evaluated by the colorimetric method. In a 10 ml flask, successively introduce 250 µl of the extract and 1 ml of distilled water. At the same time, 75 µl of a solution of $NaNO_2$ (5%), after 5 minutes, 75 µl of $AlCl_3$ (10%) and at 6 minutes, 500 µl of NaOH (1N) and 2.5 ml of distilled water were successively added. The calibration curve is established with standard solutions of quercetin prepared at different concentrations. The absorbance of the mixture is directly measured with a UV-visible spectrophotometer at 510 nm and the results are expressed in mg quercetin equivalent /gram of dry matter (EQt /100gMs).

Evaluation of the anti-radical activity of the three extracts: Qualitative analysis of the anti-radical activity was evaluated by spraying the 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution onto a silica gel TLC plate. The eluent system consisted of Ethyl acetate / Formic acid/Water (9/0.5/0.5). The appearance of pale yellow spots on a violet background indicates the anti-radical activity.

Quantitative evaluation of antiradical activity was carried out by the DPPH method.

To 10 mL of the solution of 1,1-diphenyl-2-picrylhydrazyl , 10 mg in 250 mL of ethanol and 100 µL of extract at concentrations of 50-5.0 mg/ mL were added. The activity was then measured at 517 nm in the dark after 30 minutes of incubation in a medium free from solar radiation, using a UV-visible spectrophotometer**.** The percentage of inhibition was calculated by the following relationship:

$$\%PI = \frac{Absdublanc - Absdel'extrait}{Absdublanc}^{x}100$$

White Abs: DPPH absorbance
Extract Abs: absorbance of the extract

Statistical processing: The data of this study were processed by the software EXCEL a microcommand of Microsoft and ORIGIN (version 8.5). They were used to calculate the means and standard deviations and, to plot the different histograms. To better appreciate the difference between the samples from the statistical point of view, an additional test was carried out by analysis of variance (ANOVA). The value of P is extremely low, well below the conventional significance threshold of 0.05. There are significant statistical differences between the three means of three samples with regard to yields, total polyphenol and flavonoid contents and, the 50% inhibitory concentration.

RESULTS AND DISCUSSION

The parameters for evaluating the maceration of the different samples are recorded in Table I.

Table 1. Extraction yield in percentage

Samples	Yield (%)	Maceration time (h)
E1	4.80 ± 0.5900	24
E2	5.06 ± 0.0432	48
E3	7.08 ± 0.2673	72

The yields obtained during the maceration of the pericarps of the mangosteen fruits garcinia gaertn in pure ethanol over time are mentioned in Table I. The standard deviations of 0.5900; 0.0432 and 0.2673 of different yields suggest that the individual values hardly deviate from their respective means. This indicates a low dispersion of the values. The results are almost homogeneous and close to the mean and show the existence of a harmony in the maceration process thus justifying their importance for practical applications and future analyses. Also, the yield of the sample E3 > E2>E1. The extraction duration has a significant impact on the yield.

Chemical screening: Table II shows the results obtained during the chemical screening of mangosteen pericarps extracted at different times.

Table 2. Chemical screening results

Families chemicals	E1	E2	E3
Tannins	+	+	+
flavonoid (flavanols)	+	+	+
Leucoanthocyanins	+	+	+
Alkaloids	+	+	+
Anthocyanins	+	+	+

+ presence

It emerges from this analysis that the three samples contain a multitude of phytoconstituents with a strong predominance of polyphenolic compounds. Indeed, the pericarps of fruits rich in polyphenolic compounds possess a wide range of biological activities in vitro linked to their reducing character and their affinity for proteins and metal ions. They are able to protect the body against myocardial infarction or coronary atherosclerosis which are

associated with high levels of LDL cholesterol circulating in the blood (Gouollaly *et al*., 2020).

Thin layer chromatographic analysis of extracts E1, E2 and E3: Figure 1 below shows the chromatographic profile of extracts E1, E2 and E3. This profile reveals a series of spots of different fluorescence obtained after spraying the plate with Neu and visualization at 366 nm. These spots could reflect the presence of several chemical families. Based on the literature data provided by (Wagner and Blat, 1993). The following observations can be made: The very intense Green-fluorescent fluorescences at frontal retentions (0.6*; 0.75 and 0.8) could not be attributed to derivatives of a flavonoid stricto and lato nor to phenolic acids but to another group of chemical family such as xanthone (Figure 1). (*): very intense (majority).

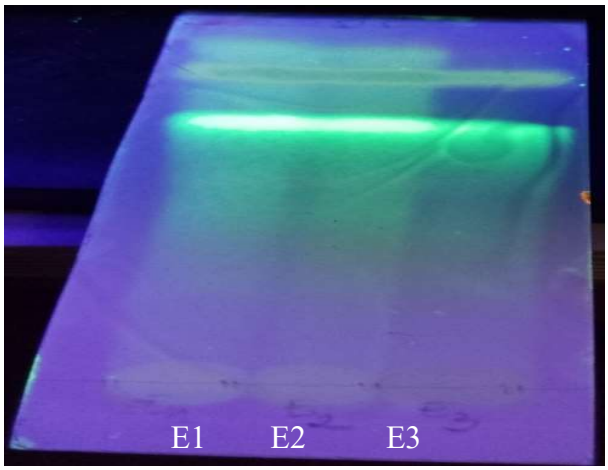

Figure 2. Chromatographic profile of pericarp extracts

Total Polyphenol and Flavonoid Contents of the Three Ethanolic Extracts: The calibration curves for the dosage of total polyphenols and flavonoids gave respectively the following equation lines: Y = 3.9089x + 0.1257 with a correlation coefficient R^2 = 0.9989 and Y = 1.6954x + 0.2816 with a correlation coefficient R^2 = 0.9955. The total polyphenol contents are expressed as mgEAG /100g Ms of the different extracts of the pericarps and those of the total flavonoids are expressed as mgQt /100g Ms (Cao *et al*, 2020). These results show that the PPT contents vary in the different pericarp extracts. The determination of total polyphenols by spectrophotometry showed that samples E1, E2 and E3 contain respectively (2033.06±12.57) mgEAG /100gMs, (2188.86±14.58) mgEAG /100gMs and (1796.86±06.48) mgEAG /100gMs. Overall the variability is consistent. The highest mean in PPT contents was observed at the level of extract E2. This sample reports a large variability in terms of standard deviation, which could require further investigation to understand the reasons for this dispersion. On the other hand, the E3 extract is more consistent with a low standard deviation, this proves that the process is stable or a very homogeneous source of polyphenol. These results can guide in decision-making for the selection of extracts from raw materials or treatments to maximize the total polyphenol content. Further analyses could be performed to explore the reasons for the observed variability. Similarly, the total flavonoid contents (1234.81± 0.750) mgEQt /100gMs; (1241.42±0.343) mgEQt /100gMs and (977.17 ±0.474) mgEQt / gMs follow the same trend as that of total polyphenols. These contents could be justified by very intense fluorescences highlighted by TLC (Figure I).

Evaluation of Anti-Radical Activityof the three Extracts of the pericarps of mangustan fruits

Qualitative evaluation: Qualitative visualization of the free radical scavenging capacity of the extracts shows yellow spots on a purple background, characteristic of the reduction of DPPH by antiradical substances in the extracts. This high activity is explained by the presence of polyphenols and flavonoids contained in these extracts highlighted on a thin layer.

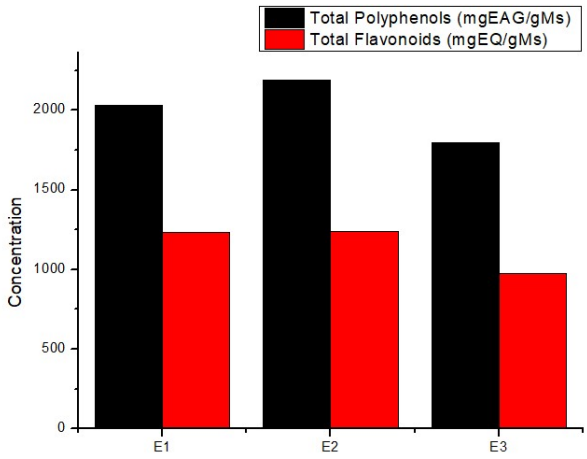

Figure 3. Total polyphenol and total flavonoid contents of mangosteen fruit pericarp extracts

Indeed, the green-fluorescent spots highlighted by TLC reflect the presence of xanthones. A molecule used as an antioxidant food supplement.It is worth noting that xanthones are powerful free radical scavengers in fruits. They protect lipids and vital cells against µoxidative damage, participate in the prevention of coronary heart disease, and exhibit antiproliferative or anticancer activities (Gouollaly *et al*, 2020).

Quantitative evaluation e: Figure 6 represents the anti-radical activity of the extracts. It shows that the three samples have an anti-radical effect against the DPPH radical with respective 50% inhibitory concentrations (IC50) of (4.15±0.1472) µg /ml; (4.22±0.1072) µg/ml and (3.70±0.1633 µg/ml). Extract E3 inhibits free radicals better than E1 and E2. This free radical reduction capacity could be explained not only by high levels of polyphenols and flavonoids present in the extracts but also by the presence of a particular molecule called xanthone highlighted by chemical screening and thin layer in the different extracts. These statements are similar to those of Laroche, 2005 in his book entitled Mangoustan: un fruit santé aux effets surpriss.

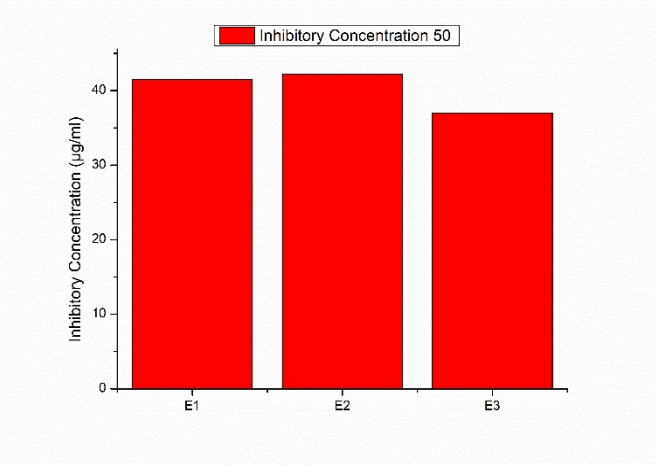

Figure 4. Inhibitory concentation 50% of the extracts

The latter reveals that the Mangosteen pericarp contains xanthones, a family of polyphenols known for their antioxidant properties superior to those of vitamin E. This statement is consistent with that of Hossakatte et al, 2020which indicates that Phenolic compounds and flavonoids present in the fruit are responsible for the antioxidant properties, including xanthones and benzophenones. Other studies have shown that mangosteen, a xanthone present in mangosteen extract, is able to both protect the skin from the harmful action of free radicals preventing skin aging and to remedy damage with a repairing effect on the skin after oxidative aggression (Mario Abate et al, 2021, Muanda, 2011).

CONCLUSION

In this work the maceration in ethanol of the dried pericarps of *Mangosteen fruits Garcinia Gaertn* was the subject of the determination of the yield, the chemical characterization and the evaluation of the anti-radical power of the extracts to evaluate the impact of the duration of this method on these parameters. The results obtained showed that overall the variability is consistent. The yield of sample E3 > E2> E1, this means that the extraction duration has an impact on the yield. In addition, the contents of total phenolic compounds and flavonoids are quite high in extracts E1 and E2. The anti-radical evaluation by TLC and by the DPPH method revealed the inhibition capacity in the three extracts. But, extract E3 showed a strong activity than that of E1 and E2. This strong activity can be attributed to the families of phenolic compounds highlighted by chemical screening and thin layer chromatography. It is assumed that the longer the maceration takes, the more new biomolecules appear. Further investigations can be carried out by separation methods to differentiate polyphenols.

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