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Evaluation of secondary metabolites followed by antioxidant dosage of *Flacourtia jangomas* fruits from Congo

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Abstract
The study carried out on the fruits of *Flacourtia jangomas* (Lour.) Räuschel enabled us to chemically evaluate the major families of secondary metabolites, followed by an assay of their antioxidant potential. The methodology adopted in this study consisted of chemical screening and TLC to identify the major families of secondary metabolites, followed by determination of total polyphenols and flavonoids, and evaluation of free radical scavenging activity.
Results showed that *F. jangomas* fruits contain tannins, leucoanthocyanins, free flavonoids and reducing compounds.
Thin-layer chromatography revealed the presence of polyphenols and flavonoids, where quantitative analyses yielded on the one hand: 1111.164; 327.338 and 704.477 mg EAG/g DM of total polyphenols on aqueous, hydroalcoholic and alcoholic extracts respectively; and on the other hand, 32.57; 26.32 and 52.79 mg ECt/g DM of total flavonoids on aqueous, hydroalcoholic and alcoholic extracts respectively.
Evaluation of antioxidant potential revealed strong antiradical activity, with inhibitory concentrations of 13.66 and 19.69 mg/mL in alcoholic and hydroalcoholic extracts.

Keywords: Antioxidant, chemical evaluation, extracts, *Flacourtia jangomas*

Introduction
Research into the phytochemical or pharmaceutical properties of foods has received a great deal of attention in recent years in different parts of the world, due to its relevance in the discovery of health-promoting foods. Fruits are recognized as one of the most valuable sources of phytochemical molecules, due to the presence of bioactive compounds such as alkaloids, terpenoids, tannins, saponins and polyphenols [1]. The most common polyphenols found in foods are flavonoids and phenolic acids [1]. These bioactive compounds are produced by the secondary metabolism of various plants. The value of these molecular substances lies in the fact that they have a definite physiological action on the human body [2].
Epidemiological studies reveal a positive association between vegetable and fruit intake and a reduction in cardiovascular disease, as well as certain cancers [3]. These molecules (Antioxidants) constitute the class that today appears to be the key to longevity and our allies in the fight against modern diseases [3]. These are protective elements that act as free radical scavengers, where free radicals are produced daily by the body. This problem, caused by oxidative stress, is described as a crucial etiological factor involved in various chronic human diseases [4]. This oxidative damage is achieved by free radical attack on various biomolecules, in particular proteins, lipids and DNA, ultimately resulting in cell degradation and death, leading to oxidative stress which contributes to accelerated cellular ageing processes [5].
The Congo, one of the countries of Central Africa, is rich in biodiversity of fruit trees, the fruits of which are sometimes under-utilized. These fruits, which belong to both the domestic and spontaneous flora, grow in certain departments of the Congo and deserve to be explored in search of the medicinal molecules that once served as an indispensable means of medicating the population [6].
Currently, research and development activities on little-known fruit species are becoming a priority in developing countries [7]. These research activities will enable a better understanding of phytochemical molecules and the fact that they are locally abundant but globally rare. Scientific information and knowledge about them is limited [8]. In view of the many difficulties encountered by the populations of the world and of the Congo in particular, it

is in this context that we proposed to evaluate the secondary metabolites followed by the antioxidant potential of extracts (aqueous, hydro-ethanolic and ethanolic) of the fruit of the *F. jangomas* (Lour.) Räuschel plant.

Materials and Methods

Plant material

The plant material (Figure 1), consisting of fruits of the jangomas species used in the present study, comes from the Kinzaba village in the Bouenza department, Congo Brazzaville. They were harvested in Mr TOBI-N'DZABA's experimental field.

Fig 1: Plant material (Source: photo taken in the laboratory)

Chemical reagents

The following solvents were used for identification and determination of *F. jangomas* secondary metabolites: hydrochloric acid, iso-amyl alcohol, magnesium copaux, 10% sulfuric acid, 20% ammonia, iron chloride, Fehling's liquor and chloroform.

Ethanol, ethyl acetate, formic acid, water, DPPH, 10% KOH, 2-aminoethyldiphenylborate polyethylene glycol 400 (PEG 400), methanol.

Methods

Preparation of solvent extracts

50 g of fresh plant material (FVM) were weighed for each extract prepared. We then added 500 mL of solvent to the FVM: water for the aqueous extract (EA), water/ethanol for the hydroethanol extract (EHE) in the proportions (1/1, v/v) and alcohol for the ethanol extract (EE). The mixture was then transferred to a magnetic stirrer Fisherbrand, hotplate and Magnetic stirrer basic serie, Model Isotemp; Germany and stirred for 48h for EE, or left to macerate for 48h for EA and EHA, before being filtered using Wattman filter paper, then evaporated using a rotary evaporator model RE-201D. The filtrate obtained was concentrated to give a sticky concentrate, which was kept cool (+4 °C) for further analysis.

Chemical analysis

Identification of major chemical families

Two methods were used to identify the major chemical families of F. jangomas (Lour.) Räuschel fruits:

- Chemical screening, and;
- Thin layer chromatography.

Chemical screening

Chemical screening is a technique used to highlight the presence of different chemical families contained in a plant. Eight families were sought: alkaloids, tannins, reducing sugars, free flavonoids, anthocyanins, leucoanthocyanins, anthracene derivatives and saponosides. Detection tests were carried out according to the methods described by Békro *et al* [9].

Thin layer chromatography (TLC)

Quantitative identification of substances with antioxidant activity was carried out by thin-layer chromatography using the "bioautography" method described by Gangopadhyay *et al*., [10] where antioxidant activity was revealed by DPPH according to Takao [11]. TLC was carried out in the normal phase on Merck silica gel chromatography plates 60F254 on a 20 cm x 20 cm aluminum foil support with the ethyl acetate/formic acid/water solution in proportions (8/1/1) and (9/0.5/0.5). The chromatogram obtained was revealed by sputtering with 10% Neu and KOH solutions. The Neu solution was obtained from a mixture of 1% (w/v) 2-aminoethyldiphenylborate and 5% (w/v) polyethylene glycol 400 (PEG 400) solutions in methanol.

Plates were observed after sputtering (Neu and KOH) under UV light at 365 nm.

Determination of antioxidants

Determination of total polyphenols

The total polyphenol content of the various *F. jangomas* fruit extracts was determined using the Folin-Ciocalteu method.

Using test tubes, so-called "daughter" extracts were prepared from the mother extracts at different concentrations, with 0.1 mL of each extract being added to 0.9 mL of distilled water in each tube. For each extract prepared, concentrations varied in decreasing order as follows: from 69.5 to 4.34 mg/mL for the aqueous extract; from 57 to 3.57 mg/mL for the hydroethanol extract; and from 67 to 4.19 mg/mL for the ethanol extract. To each preparation, 0.9 mL of 1N Folin-Ciocalteu reagent was added, followed immediately by 0.2 mL of sodium carbonate solution (20% Na_2CO_3). The resulting mixture was incubated at room temperature (25 °C) for approximately 40 minutes, protected from light.

Absorbance was measured using a VWR brand spectrophotometer, UV-3100 PC spectrophotometer ECN: 634-6042, China at 725 nm against a methanol solution used as a blank. It should be noted that a calibration line was previously performed with gallic acid under the same conditions as the samples to be analyzed.

The results obtained were expressed in mg gallic acid equivalent per gram of dry matter (EGA/g Ms).

Determination of total flavonoids

The total flavonoid content of the various *F. jangomas* fruit extracts was obtained using Aluminium Trichloride (AlCl3) [11]. A solution with a concentration of 8.125 mg/mL was prepared beforehand. 250 µL of each extract (Aqueous, hydroethanolic and ethanolic), 1 mL distilled water and 75 µL sodium nitrite (5% $NaNO_2$) were successively introduced into a 100 mL flask. The mixture was left to stand for 5 minutes before adding 75 µL of aluminum trichloride (10% $AlCl_3$). After 1 minute, 500 µL of 1N sodium hydroxide (NaOH) and 2.5 mL distilled water were successively added to the mixture.

A calibration curve was constructed using catechin standard solutions prepared at different concentrations. Absorbance was measured with a UV-visible spectrophotometer at 510 nm, and results were expressed in mg catechin equivalent per gram of dry matter (ECt/g Ms).

Evaluation of the anti-free radical activity of different extracts

DPPH method

Evaluation of the anti-free radical activity of the various extracts (Aqueous, hydro-ethanolic and ethanolic) was based on the DPPH (1, 1-diphenyl-2-picrylhydrazyl) method. 10 mL of the latter's ethanolic solution (10 mg DPPH in 250 mL ethanol) and 100 µL of each daughter extract (prepared as in the total polyphenol assay section) were mixed in EDTA glass tubes. After 30 minutes, incubation in the dark, free radical scavenging activity was measured by spectrophotometer at 517 nm [12].

Percentage inhibition was calculated using the following relationship:

$$\% I = \frac{D.O\ blank - D.O\ extract}{D.O blank} x100 \quad (1)$$

With D.O blank: 0,879 D.O extract (cf. tables I, II et III).

50%Inhibitory concentration

The IC_{50} parameter is defined as the substrate concentration that causes the loss of 50% of DPPH activity. Antioxidant power is determined so that a given concentration of extract neutralizes 50% of the DPPH radical. To enable extracts to be compared with each other, this index is obtained either by deduction from the curves of the variation in inhibition percentage I%, or calculated graphically using the formula of regression of inhibition percentages as a function of different extract concentrations tested with Origine Pro 8 software. The value of anti-free radical activity, such as y = 50%, corresponds to the inhibitory concentration IC_{50} of the extract studied [12, 13, 14].

It should be remembered that the smaller the IC_{50} value, the greater the antioxidant activity of the extract [15].

The results expressed as IC_{50} were deduced from the data presented from the variation of the percentage inhibition I % as a function of the concentration of each extract.

$$CI50 = \frac{C(dosage)\ x\ 50\%}{\%I\ of\ the\ extract}$$

With:

C (dosage): concentrations used for the assay of total polyphenols and flavonoids;

% I: inhibition percentages for each extract.

Results and discussion

Total yield of crude fruit extract

The extraction yield of glutinous residues from the crude extract obtained from *F. jangomas* fruits is shown in Table I.

Table 1: Extraction yield

Extract type	Extraction yield (%)
Aqueous extract	12,00
Hydroethanol extract	12,60
Ethanolic extract	18,96

Extraction yields vary from one plant to another, and depend on numerous parameters such as the physico-chemical characteristics of the solvents used, particularly their polarity, the parts of the plant material used, the method, and even the conditions under which extraction was carried out.

For example, Senthil Kumar *et al*. [16] reported extraction rates of 10.42% and 12.33% respectively for ethanolic and aqueous extracts from *Flacourtia jangomas* leaves, while our work on F. jangomas fruit reported a similar value of 12% for the aqueous extract, and very high values of around 12.6% for the hydro-ethanolic extract and 18.96% for the ethanolic extract.

Chemical analysis

Chemical screening of *Flacourtia jangomas* L fruits

The results of the tube reactions obtained are shown in Table II. These results revealed that *Flacourtia jangomas* L fruits contain free flavonoids, tannins, leucoanthocyanins and reducing sugars. Alkaloids, saponosides, anthocyanins and anthracene derivatives were absent.

Table 2: Chemical screening of fruits of *F. jangomas* L

Secondary metabolite families	Results
Alkaloids	-
Tannins	+
Free flavonoids	+
Anthocyanins	-
Leucoanthocyanins	+
Saponosides	-
Reducing compounds	+
Anthracenic derivatives	-

Legend: Present: +; Absent: -

Results obtained by Bimal Dutta *et al*. [17] on the same fruits revealed the presence of flavonoids, phenols, tannins, terpenoids, saponins and an absence of alkaloids on shade-dried fresh fruits, using methanol extract as solvent. The difference in results could be justified by the type of extraction (Method) and solvent used.

Nguyen Duy Tan *et al*., [18], on the other hand, revealed the presence of anthocyanin, alkaloids, β-carotene, flavonoids, tannins, saponins and phenolic compounds in the ethanolic extract on the same type of fruit.

Senthil Kumar *et al*., [16] instead revealed the presence of glycosides, phytosterols, alkaloids, glycosides, polyphenols, flavonoids, proteins and amino acids in the ethanolic extract. With the exception of phytosterols and alkaloids, the remaining compounds were founds in the aqueous extract. Terpenoids, gums and mucilages, oils and fixed fats were absent in both ethanolic and aqueous extracts.

Thin Layer Chromatography (TLC)

TLC results are shows in Table III. The spots obtained may reflect the presence of several families of chemical compounds.

Table 3: Chromatographic profile of *F. jangomas* fruit extracts

Extraction process	Extracts	Number of spots	Rf (cm)	Fluorescence at 365 nm	Compound families
Maceration in ethanol	Ethanolic extract	1	0,5	Dark Blue (DB)	Gallic acid derivatives
		2	0,35	Green (G)	-
		1	0,78	Blue-white (BW)	Flavonoid (Flavone)
		1	0,33	Orange-Yellow (OY)	Flavonoids (Flavonol)
		1	0,10	Light Green (LG)	Polyphenols (hydroxy-cinnamic derivatives)
Maceration in water + alcohol	Hydroethanol extract	1	0,5	Dark Blue (DB)	Phenolic acids (Gallic acid derivatives)
Maceration in water	Aqueous extract	3	0,33	Blue-White (BW)	Flavonone (luteolin derivative)
			0,73		
			0,21		

Legend: frontal ratio (Rf)

The ethanolic extract revealed six (6) spots, two of which were green with an Rf of 0.35 cm, reflecting a family of unidentified compounds. One spot each, dark blue (Rf = 0.5 cm), bluish-white (Rf = 0.5 cm), orange-yellow (Rf = 0.5 cm) and light green (Rf = 0.5 cm), reflected gallic acid derivatives, flavones, flavonols and polyphenols (hydroxy-cinnamic derivatives) respectively. As for the aqueous extract, it revealed three (03) spots of bluish-white coloration at distances of Rf = 0.21; 0.33 and 0.73 cm) reflecting a single family of compounds, Flavonones (luteolin derivative). Finally, the hydroethanol extract also revealed a single dark-blue spot at 0.5 cm corresponding to phenolic acids (such as gallic acid derivatives).

Quantitative analysis

Determination of total polyphenols and flavonoids

Typical calibration curves for PPT and FVT are shows in figures 2 and 3.

Calibration curves for PPT assays

The calibration curve was established using gallic acid as the reference (Figure 2). Results were expressed as mg gallic acid equivalent per gram of dry matter (mgEAG/100 gMs). The calibration curve was established with a correlation coefficient $R^2 = 0.9989$.

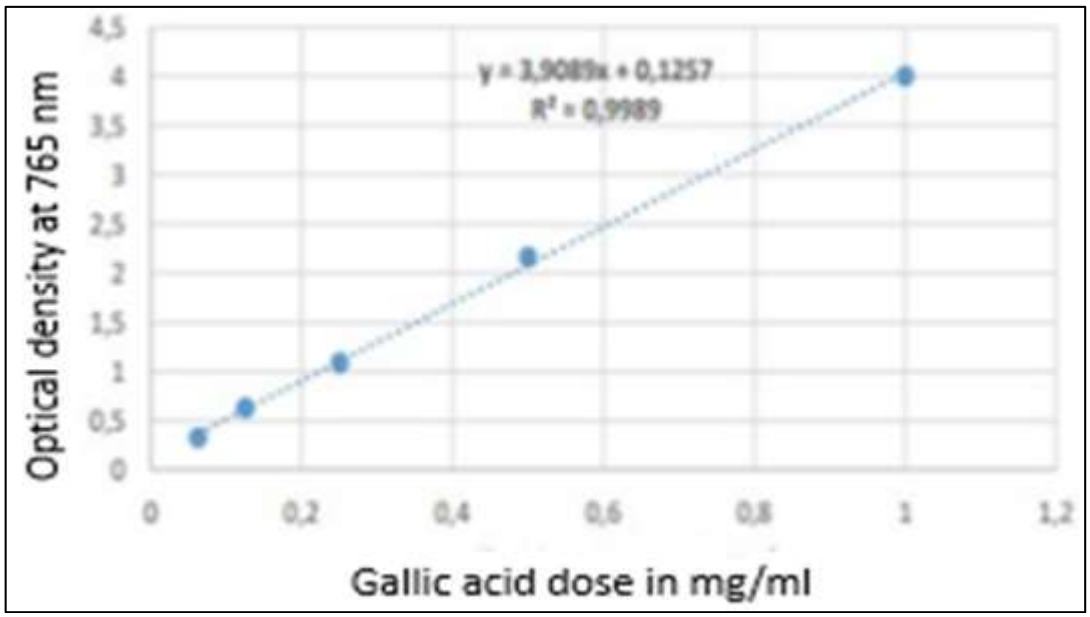

Fig 2: Total polyphenols calibration curve

Calibration curves for FVT assays: Catechin was used as the reference compound for this curve. The curve was established with a correlation coefficient $R^2 = 0.9974$ (Figure 3). The results obtained were expressed in mg catechin equivalent per gram of dry matter (mgECt/100 gMs). The results of quantitative spectrophotometric analysis of polyphenolic compounds and flavonoids in the aqueous, hydroethanol and ethanol extracts are shown in Figure 4.

This quantitative analysis of total polyphenol compounds showed that the aqueous extract was quantitatively richest (1111.164 mgEAG/g DM), followed by the ethanol extract (704.477 mgEAG/g DM) and finally the hydroalcoholic extract (327.338 mgEAG/g DM). On the other hand, the ethanolic extract was richest in total flavonoids (52.79 mgECt/g DM); followed by the aqueous extract (32.57 mgECt/g DM) and finally the hydro-ethanolic extract (26.32 mgECt/g DM). The assay results showed that the proportions of polyphenols were significantly higher than those of flavonoids, meaning that in all extracts, non-flavonoid polyphenols were in excess. These observations suggest that the variation in flavonoids is not relative to the variation in polyphenols (Figure 4). These same observations concur with those of numerous previous studies, such as those by Bimal Dutta *et al*., [17] whose total polyphenol content was 20 mg/g EAG and total flavonoid content, 2 mg/g EQt for methanoic extract.

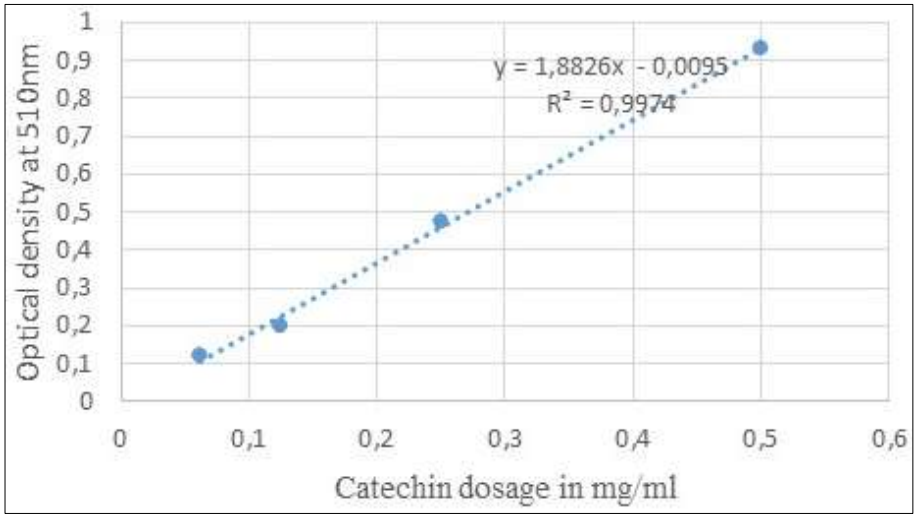

Fig 3: Total flavonoids calibration curve

In an earlier study on the ethanolic extract of *Flacourtia jangomas* fruits, Nguyen Duy Tan *et al*., [18] obtained a total flavonoid content of 65.96 mg EQ/100g DM and a total polyphenol content of 456.32 mg AEG/100 g DM.
Sarmaa A *et al*., [20] reported values of 390 mg EAG/100 g and 6.66 mg QE/100 g dry matter respectively for total polyphenols and total flavonoids in methanoic extract.) These results are lower than those obtained previously. The work carried out by Barbhuiya RI *et al*., [19] on the other hand, on Indian coffee as well as that of Malaysia on fruits of the same plant (*F. jangomas*) revealed a very high phenolic content, respectively 3,297 mg EAG/100 g and 2,507.41 mg EAG/100 g.

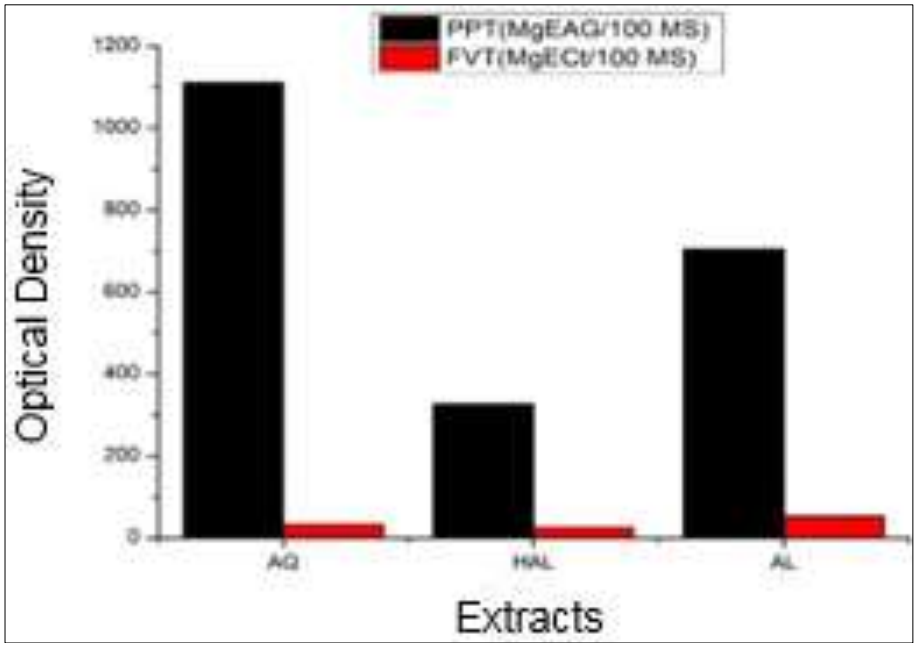

Fig 4: Total polyphenol and flavonoid content

Anti-free radical activity of various extracts
Qualitative assessment
The qualitative visualization of the free radical scavenging capacity of the extracts (Aqueous, hydroethanolic and ethanolic) is shown in figure 5. This figure shows yellow spots on a violet background, characteristic of DPPH (radical) reduction by anti-radical substances in the extract. The polyphenolic compounds responsible for this activity are attributed to flavonoids, since these are known to be powerful free radical scavengers.

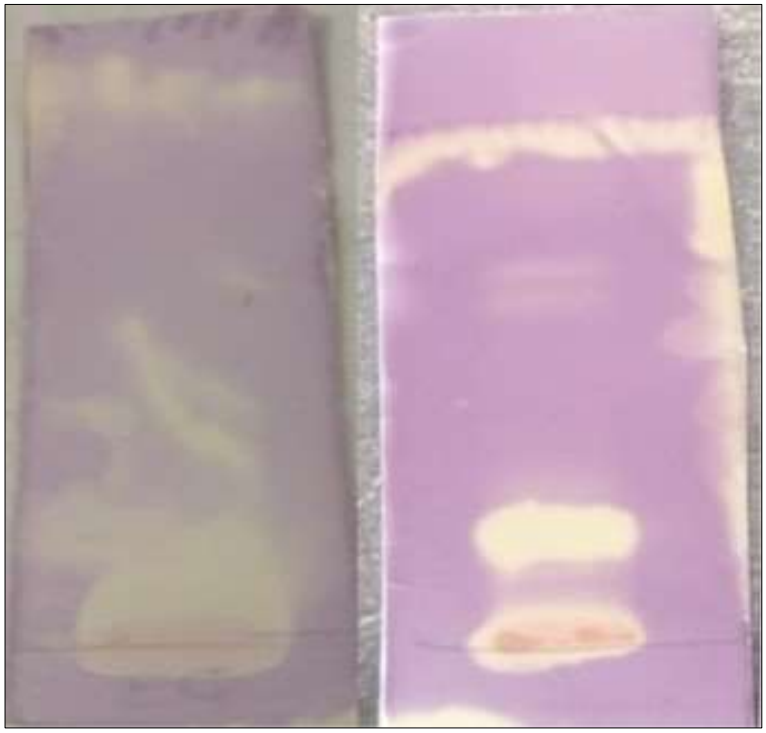

Fig 5: Anti-free radical activity of the extract

Quantitative evaluation

Percentage of DPPH radical inhibition: The results of the anti-free radical activity of the various extracts on DPPH are presented in Table IV. These tables show that at low concentrations of the aqueous, hydro-ethanolic and ethanolic extracts, inhibition percentages of 10.810%, 18.090% and 26.500% respectively. At higher concentrations, however, the percentages were 70.190%, 80.430% and 90.320% respectively. It can be seen that anti-free radical activity values increase as a function of concentration in each extract.

Table 4: Anti-free radical activity of aqueous, hydroethanolic and ethanolic extracts of *F. jangomas*

	Aqueous extract				
Concentration (mg/mL)	67.000	33.500	16.750	8.380	4.190
Optical density (O.D)	0.262	0.490	0.641	0.698	0.784
Percentage of inhibition (%)	70.190	44.250	27.070	20.590	10.810
	Hydroethanol extract				
Concentration (mg/mL)	57.00	28.500	14.250	7.130	3.560
Optical density (O.D)	0.172	0.306	0.573	0.643	0.720
Percentage of inhibition (%)	80.430	65.190	34.810	26.850	18.090
	Ethanolic extract				
Concentration (mg/mL)	69.500	34.750	17.380	8.690	4.340
Optical density (O.D)	0.085	0.151	0.408	0.599	0.646
Percentage of inhibition (%)	90.320	82.820	53.580	31.850	26.500

Nguyen Duy Tan *et al*., [19] on the ethanolic extract of *F. jangomas* fruits obtained an antioxidant capacity of around 88.65%.

50% inhibitory concentration

Quantitative results for the free radical scavenging activity of *F. jangomas* fruit extracts (aqueous, hydroethanolic and ethanolic) were also expressed using the IC_{50} parameter. The lower the IC_{50} value, the greater the antioxidant activity of a compound (figure 6). We note that the ethanolic extract has a remarkable free radical scavenging effect on the DPPH radical, followed by the hydroalcoholic extract with 50% inhibitory concentrations (IC_{50}) of 13.660 and 19.690 mg/mL respectively. In contrast, the inhibitory concentration of the aqueous extract was 20.330 mg/mL.

These low values for the 50% inhibitory concentration (IC_{50}) of the ethanolic and hydro-ethanolic extracts revealed a greater antioxidant power than that of the aqueous extract, and this could be explained by the fact that alcohol and the alcohol/water mixture would be the best extraction solvent for this study.

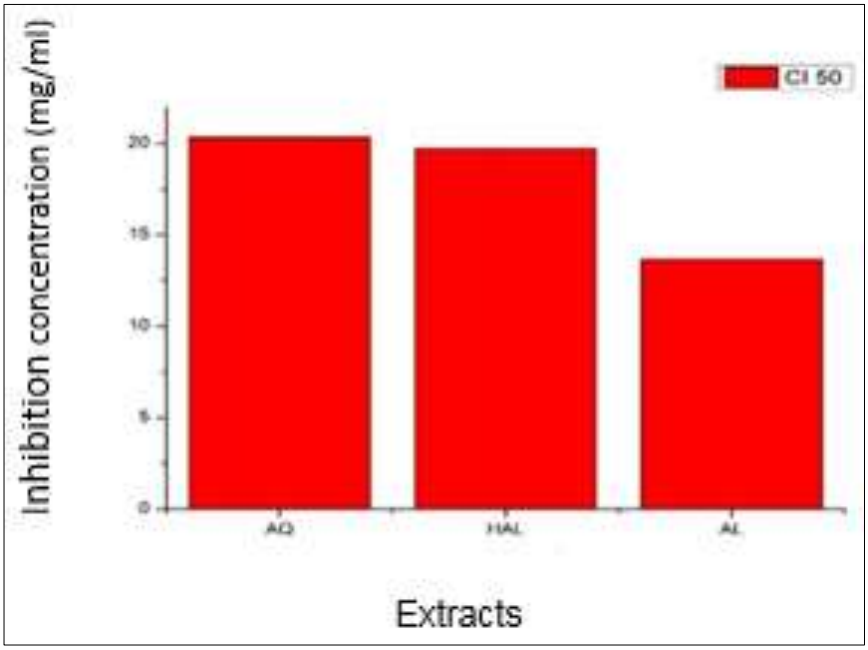

Fig 6: Anti-free radical activity in extracts

Studies by Sajeesha Sasi *et al*., [21] reported an inhibitory concentration (IC_{50}) on F. jangomas fruit of 1.144 mg/mL in methanoic extract, a much lower value than those obtained in our work.

Conclusion

The aim of this study has been achieved. This study shows that *F. jangomas* fruits contain high levels of polyphenols and carbohydrates. However, flavonoid levels are very low.

Phytochemical screening revealed the presence of free flavonoids, tannins, leucoanthocyanins and reducing sugars. Alkaloids, saponosides, anthocyanins and anthracene derivatives were absent.

Thin-layer chromatography confirmed the presence of total flavonoids, in particular flavones derived from luteolin, and polyphenols. Spectrophotometric determination gave total polyphenol concentrations of 1111.164 mgEAG/g MS for the aqueous extract, 327.338 mgEAG/g MS for the hydroethanol extract and 704.477 mgEAG/g MS for the ethanol extract. For total flavonoids, we obtained 32.57 mgECt/g MS in the aqueous extract; 26.32 mgECt/g MS in the hydroethanol extract and 52.79 mgECt/g MS in the ethanol extract.

Evaluation of antioxidant potential revealed significant free radical scavenging activity for the ethanolic extract, followed by the hydroethanolic extract with inhibitory concentrations of 13.66 mg/mL and 19.69 mg/mL.

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Conflicts of Interest

No conflict on this article.

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