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Antioxidant pure secondary metabolites from the root bark extract of *Sapium ellipticum* (Hochst) Pax

Olga Nana[a*], Jean Momeni[a], Fadimatou spouce Abdourahman[a], Martin Benoît Ngassoum[b].

[a] *Department of Chemistry, Faculty of Science, Ngaoundere, Cameroon*

[b]*National Advanced School of Agro-Industrial Sciences, Ngaoundere Cameroon.*

Scope of research

- Phytochemical screening of *Sapium ellipticum* root bark extracts
- Investigation of qualitative DPPH assay using Thin Layer Chromatography (TLC) of *Sapium ellipticum* root bark extracts
- Evaluation of antioxidant activity using DPPH radical scavenging and oxidative degradation of β-carotene of different *Sapium ellipticum* extracts.
- Isolated and purification of one flavonoids and two terpenoids from ethyl acetate *Sapium ellipticum* extract using colum chromatography (CC) m n
- Evaluation of antioxidant activity using DPPH radical scavenging and oxidative degradation of β-carotene of pure flavonoids and pure terpenoid.

Abstract: *In this study, two terpenoids and one flavonoid were isolated from Sapium ellipticum (Hochst) Pax root bark labelled TS1, TS2 and FS. Extracts and these pure compounds were tested for their antioxidant potential by using DPPH radical-scavenging and oxidative degradation of β-carotene. The total amount of phenolic compounds in the extract was determined in terms of gallic acid equivalent and in terms of quercetin equivalent. The isolated compounds exhibited strong antioxidant activities than crude extracts with IC_{50} values of 1.59 10^{-3}, .2.6 10^{-3} and 7.9 10^{-4}mg. mL^{-1} respectively compared to crude ethyl acetate extract with IC_{50} values of 2. 10^{-2}mg. mL^{-1}, crude EtOAc/EtOH extract with IC_{50} values of 5.4.10^{-2}mg. mL^{-1} and crude MeOH extract with IC_{50} value of 11.02.10^{-3}mg. mL^{-1} but less than BHT value IC_{50} 3.36µg. mL^{-6}taking as control. The high activities of these plant extracts suggest that it is a good source of natural antioxidant and may contain new secondary metabolites with exceeding structures for many formulations against countless diseases in general and in particular premature ageing due to the presence of free radicals in the human body.*

Keywords: *Sapium ellipticum, antioxidant activity, free radical scavenging power, degradation of β-carotene.*

1. Introduction

Plants will always remain a vast field of investigation in search of bioactive molecules. Plants with therapeutic activities compared to some modern drugs have failed to yield satisfactory results, give good results and reduced side effects (Ighodaroet al. 2017). Although some are toxic, their studies are generally made to determine the degree of toxicity to integrate it into the ethnobotanical investigation.Many secondary metabolites isolated from medicinal plants are useful as active compounds and serve as drug leads for new bioactive molecules. About human health, when the overproduction of reactive species exceeds the antioxidant capacity of cell defence mechanisms, oxidative stress occurs. The cause of such unsteadiness in the body can be xenobiotic, therefore, an increase in the amount of antioxidants is necessary. This oxidative stress and imbalance of antioxidant properties are often the cause of cancer and cardiovascular diseases (Parcheta et al. 2021).

Sapium ellipticum (Hochst) Pax is a plant in traditional medicine used for the treatment of a series of diseases. The medicinal activities of *S. ellipticum* leaves are attributed to the existence of its heterocyclic compounds/phytochemical constituents (Onukwuli et al. 2020). In the Far North of Cameroon, the population used it against malaria, intestinal diseases and abdominal pain. All part of *S. ellipticum* is used for their therapeutic effects. The decoction of leaves or stem bark of this plant has been used in Ethiopia, Congo, Cameroon and Burundi in traditional medicine to treat gastrointestinal disorders, particularly diarrhoea (Wansiet al. 2014). The roots bark and leaves together are dewormers and fight against eye pain. Root barks used as a laxative, fights against infantile splenomegaly, stuttering, cough and malaria, The stem bark fight against anaemia, fever, guinea worm, elephantiasis, rheumatic, burn treatment, eczema, dracunculiasis scurvy and stomatitis. Leaves are used against

abdominal swelling, eye diseases and mumps. Stem against wound healing, chest pain, and headache (Mwine et al. 2011;Neuwinger et al. 1994).Belonging to the Euphorbiaceae family, *S. ellipticum* also none as *Shirakiopsiselliptica*, is a shrub or small monoecious three, strongly branched, reaches 15 to 45 m high, it has no branch for a maximum of 12m. From *S. ellipticum* some secondary metabolites such asterpenoids, flavonoids, alkaloids, quinones, anthocyanins, saponins, steroids, and tannins having applications in phytotherapy, were isolated (Mbayoet al. 2016;Kisangauet al. 2009). In the same direction,*S. ellipticum* extracts appearing to have the phyto-proficiency, protects against membrane peroxidation and improves the activities of some first line antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT)*in vivo* in the face of overwhelming reactive species, probably due toits array of bioactive compounds (α-tocopherol, amentoflavone and lupeol) which were previously identified and reported (Ighodaroet al. 2018;Edimealemet al.2013). Their study demonstrated the presence of lupeol acetate and stigmasterol in the stem bark extracts.In addition, terpenoids with antifungal potential and flavonoids with high antioxidant activities can reduce the access of oxidants and other deleterious molecules have been obtained from *S. ellipticum* stem bark. This property is due to their ability to quench oxygen-derived free radicals by donating a hydrogen atom or an electron, or to chelate redox-active metals and inhibit lipooxygenases (Ighodaroet al. 2017).

Free radicals are oxidants capable of oxidising biomolecules and lead to mutagenic changes, tissue damage and cell death. They have a significant pathological role in the development of cancer, emphysema, cirrhosis, atherosclerosis, arthritis, and others degenerative diseases (Yue et al. 2009).

Although few scientific investigations have been conducted on *S. ellipticum* some allowed toisolate triterpenes, steroids, polyketides and flavonoids (Ighodaroet al. 2017) with interesting biological activities. To the best of our knowledge, no research work concerning the isolation of antioxidant triterpenes and flavonoids from ethyl acetate extract of*S. Ellipticum* and the comparison of their antioxidant potential with other extracts (MeOH, EtOAc/EtOH) has been done on this medicinal plant studied in the present work.

2. Materials and Methods

2.1 Source of plant material

The plant samples of *S. Ellipticum* were collected in the month of December 2020, from a forest in a suburb around Ngaoundere town (Adamawa region of Cameroon). The samples (root bark) were taxonomically authenticated by Pr Mapontmesem, a botanist in the Department of Biological Sciences, Faculty of Science of the University of Ngaoundere. The samples were freed extraneous materials shade dried at room temperature and powdered using a grinder. An amount of4 kg was obtained. A Voucher specimen number SRFC/49462 of the plant is kept at the National Herbarium.

2.2 Extraction procedure

Five hundred grams of powdered *S. ellipticum* root bark were extracted at room temperature (25˚C) with 1500 mL of different extracting solvents, successively(ethyl acetate; ethyl acetate/ethanol and methanol) by maceration for three hours under mechanical agitation using an adapted agitation system apparatus which dissolving the solute in the solvent and increase heat and mass transfer coefficients. The resulting filtrate was further filtered through medium flow Whatman filter paper (No 1, pore size 11µm) and subsequently reduced in volume under reduced pressure on a Büchii rotary evaporator at 40˚C. This operation was repeated three times. The residue was then successively extracted in the same manner with ethyl acetate/ethanol (1:1) and methanol. The quantities of these extracts and their yields obtained are given in Table 1.

The extraction yields were calculated as follows:

$$Y\% = M_1/M_2 * 100,$$

where M_1 (g) and M_2 (g) are respectively the weight of extract and root bark dried powder and Y is the extraction yield. The different extracts were stored at 4°C for further investigations.

2.3 Phytochemical screening

S. ellipticum root bark extracts were subjected to preliminary phytochemical screening according to standard procedures. These extracts were tested for the presence of alkaloids, flavonoids, phenols/polyphenols, anthocyanins, quinones, saponins, sugars, triterpenoids, and tannins (Mbayo et al. 2016;Treaseet al. 1989).

2.4 Determination of antioxidant activity

2.4.1 *Qualitative DPPH assay using Thin Layer Chromatography (TLC)*

The qualitative test to DPPH consisted of using a chromatographic TLC plate on which spots of *S. ellipticum* root bark extracts were spotted and developed in the system of n-hexane-ethyl acetate and ethyl acetate-methanol system. Two hundred micrograms of dissolved extracts, were spotted on silica-gel 60 F254 plates (Merck, Germany) and were developed in adequate solvent systems for instance: EtOAc/MeOH (35:15) for EtOAc extracts; EtOAc/MeOH/H_2O (30:15:5) forEtOAc/EtOH extracts; and EtOAc/MeOH/H_2O (15:25:10) for MeOH extracts. Two plates were prepared under the same conditions, one for the antiradical test and the second to be detected with plant drug reagents to establish a relation between the antioxidant activity and the nature of the present secondary metabolites. The first plate was sprayed with a methanolic solution of DPPH (2 mg/ml) considering yellow spot on the purple background as a positive antiradical activity with butylhydroxytoluene (BHT) used as a positive control. The second plate was revealed by spraying with 50% H_2SO_4 to detect different groups of compounds (orange-yellow spots indicating polyphenolic compounds, flavonoid compounds are detected as yellow-orange colour) after the plate was visualized at UV 254 nm and UV 365 nm for other fluorescent spots) followed by heating at 105˚C in 5 min. for others(Nana et al. 2013). The odd electron is paired in the presence of a free radical scavenger, the absorption decrease and the DPPH solution is decoloured as the colour from deep purple to light yellow. The decrease of absorbance measurement is indicative of the radical scavenging power of the extract. The fast colour change is an indication of the high antioxidant potential of the extracts (table 2).

2.4.2 *Quantitative antioxidant test using DPPH radical-scavenging activity*

The antioxidant capacity was measured according to the method used by Yang with some slide modifications(Yang et al. 2021). Precisely, a stock solution of 100 µg/mL of each extract or pure organic compound was prepared by diluting 0.5 mg in 5 mL of solvent. By different dilution of the stock solution, a standard range of concentrations: 100, 80, 60, 40, and 20µg/mL were obtained. Butylated hydroxytoluene (BHT) was used as reference compound. For each concentration, assays were triplicated, the optical densities were measured using a Metertech Germany Spectrophotometer UV/vis sp 8001 at 517 nm and the antioxidant activities were evaluated using the following formula:

$$IP\% = (A_{\text{of control-A sample}})/A_{\text{of}}\text{control}) * 100,$$

where initial A is the absorbance of the control reaction and A sample is the absorbance of the test extract or purified organic compound.

2.4.3 *Quantitative Antioxidant test using β-carotene-linoleic acid*

In this assay, antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxide arising from linoleic acid oxidation (Dapkeviciuset al. 1998). A stock solution of the β-carotene-linoleic acid mixture (500 µg/mL) was prepared by dissolving 5 mg of β-carotene in 10 mL of chloroform, and 250 µL of linoleic acid and 2 g of tween 40 were added. Chloroform was completely evaporated under vacuum using Buchi rotary evaporator. Then, 1 L of aerated distilled water was added with vigorous starring to form an emulsion. Aliquots (1.5 mL) of this emulsion were then transferred into different test tubes containing different concentrations of test sample (0.1000, 0.0500, 0.0250, 0.0125, and 0.0100 µg/mL of the different extracts. As soon as the emulsion was added to each tube, the zero-time absorbance was measured after 5 minutes in a water bath at 490 nm using Metertech Spectrophotometer UV/vis sp 8001. Thereafter, the tubes were placed at 50°C in bath water for 2 h before re-measuring. A blank treatment served as the control for the spectrophotometric readings. The same procedure was repeated with the synthetic antioxidant BHT, as the reference. Antioxidant activity (AOA) was calculated using the following equation:

$$\%AOA = (A_{2H}/A_i)*100;$$

with % AOA, the antioxidant activity; A_{2H} the absorbance of β-carotene content after 2 h; and A_i the initial absorbance of β-carotene content. (Avlessiet al. 2004).

2.5 Determination of total flavonoid content

The total flavonoid content was brought up using the Dowd method adapted by Arvouet-Grant et al., (1994). In brief, 1 mL of methanolic solution of 2% aluminium chloride solution $AlCl_3$ was mixed with 1 mL of investigated ethyl acetate extract (1 µg/mL). The mixture was allowed to stand at room temperature for 15 min. with intermittent shacking. The standard curve for total flavonoids was made using quercetin standard

solution (0–100 µg/L) using the same aforementioned procedure. The absorbance of the mixture was measured at 415 nm against a blank sample without aluminium chloride using Metertech Germany Spectrophotometer UV/Vis sp 8001. The content of total flavonoids was calculated and expressed as milligrams of quercetin equivalents (QE) per gram of dry matter of the extract.

2.6 Determination of total phenolic content

The content of total phenolics (TPC) in *S. ellipticum* root bark extracts was determined by the Folin-Ciocalteu colourimetric method (Cheng et al.2009) using gallic acid as the reference standard. The absorbance was measured on the Metertech Spectrophotometer UV/Vis sp 8001 using a glass curve against a blank in the first test tube and the recovery of total phenolic compounds was expressed as mg of gallic acid equivalents (GAE) per gram of dry weight extract (mg GAE/g DW**.**

2.7 Isolation procedure of compounds from *S. Ellipticum* root bark extract

Several separations and purification methods such as thin layer chromatography, column chromatography, preparative thin layer chromatography, and recrystallization were used to isolate the pure compounds from the ethyl acetate extract of *S. ellipticum* root bark. Thin layer chromatography (TLC) was performed on silica gel precoated plates F-254 Merck and spots on the TLC plates were visualized at 254, 365 nm and after spraying with 50% H_2SO_4 followed by heating at 105˚C. Fractions of similar TLC mobility and band formation patterns were pooled together into a pre-weighed beaker and those with more than one band were subjected to another thin layer and column chromatographic procedures to obtain more pure compounds.

Specifically, the ethyl acetate extract of *S. ellipticum* root bark (15 g) was subjected to column chromatography to separate into its component. For it, we used a 2000 mL column and150 g of silica gel (60 Merck granulometry 70-230 Mesh) as the stationary phase while varying solvent with gradient of CH_2Cl_2, CH_2Cl_2-EtOAc (1:1), EtOAc-MeOH (1:1) and MeOH. combinations of increasing polarity as mobile phase. Seven fractions (F1…to F7) eluted with CH_2Cl_2 were combined based on the thin layer chromatography (TLC) analysis, a brown precipitate was obtained which after recrystallization with CH_2Cl_2/-EtOAc (15:85) (v/v) gaves a yellow organic compound labelled FS1 (10 mg). F'(3 g) from (F10….to F15) was subjected to another column chromatography over silica gel and eluted with CH_2Cl_2-EtOAc (1:1) and EtOAc-MeOH (5:95) to afford respectively two pure terpenoids labelled TS1 (8 mg) and TS2 (6 mg). Remaining fractions obtained with CH_2Cl_2-EtOAc (1:1), EtOAc-MeOH (1:1) and MeOH were kept for future investigations.

2.8 Statistical Analysis

Experiments were performed in triplicate and values were expressed as means SD and were assessed for statistical significance using the Statgraphics plus XVI.II software. Graphs were done on Sigma plot 12.0 software and correlation and regression analyses were performed using Microsoft Excel 2016. Differences between means, correlations and regressions were considered statistically significant at $p < 0.05$.

3 Results and Discussion

3.1 Extraction Yields

An efficient and effective extraction technique is decisive not only for the quality of the investigation but also for the isolation of different classes of secondary metabolites. Table 1 below shows the extractions yield of the different fractions. The high extraction yield, 25.33% belongs to the MeOH solvent which has a stronger solubility on the extractable compounds present in *S. ellipticum* root bark. The solubility phenomenon is more intense and the disruptions of root bark samples under polar solvent take place. These results concurred with previously published results (Nana et al. 2021). However, the other two extracts are not different. Ethyl acetate extract and ethyl acetate extract from ethanol extract are not different when compared to their amount of secondary metabolite content. The extract yields of *Sapium ellipticum* obtained with different solvents are in the order: methanol > ethyl acetate/ethanol > ethyl acetate, this classification is that of the ascending order of polarity of the solvents. The results obtained in Table 1 below is in accordance with the fact that *S. ellipticum* is a plant with more polar secondary metabolites than a polar one (Mbayo et al. 2016). This explain and justify the different extraction yield obtained.

Table 1 Total phenolic, total flavonoid, and extraction yield of different extracts from *S. ellipticum* root bark

Plant extract	Extraction yield (%)	Total flavonoid (mg quercetin equivalent/g dry sample)	Total phenolic (mg gallic acid equivalent/g dry sample)
MeOH	25.33±0.77	38.12±1.85	71.02±1.36
EtOAc/EtOH	15.11±1.01	27.82±1.44	60.33±1.03
EtOAc	13.22±0.26	28.52±0.97	45.62±1.22

3.2 DPPH staining procedure and phytochemical screening by TLC

After a qualitative study of the free radical scavenging capacities of the different extracts of *S. ellipticum* root bark (Table2), in order to know the antioxidant potential of these extracts, two method based on the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene/linoleic acid degradation were carried out. A compound with the ability to scavenge or deactivate radicals, generally, by donating an H-atom or an electron to the radicals, is none as an antioxidant compound. DPPH is a stable radical with a deep purple colour whose reaction with other radicals, reducing agents, or compounds capable of HAT (hydrogen atom transfer) results in a loss of colour at 515 nm; the DPPH radical reacts with its two electrons and hydrogen donors (Apaket al. 2022). Table2 shows the results of free radical scavenging capacities of the extracts which indicate that ethyl acetate extract of *S. ellipticum* is more antioxidant than the other two extracts but less than BHT taken as control. In addition, all the investigated extracts and BHT at various concentrations prevented the bleaching of β-carotene to different degrees. The β-carotene in this model system undergoes rapid decolourization in the absence of an antioxidant, because of the couple oxidation of β-carotene and linoleic acid, which generate free radicals (Bektas et al. 2005).

Table2 Thin layer chromatographic results of rapid anti-free radical and antioxidants tests

Plant / reference	Root barks extracts	Antioxidant test (maintenance of the colouration of the β-carotene after 3 h).	Free radical power (Discolourization time (DPPH•)
Sapium ellipticum	MeOH	180 min	<20s
	AE	190min	<10s
	AE/EtOH	120 min	<30s
BHT	BHT	200 min<	<5s

It appears from the Table 2 above that the polar extracts exhibited strong activity compared to

non-polar extracts, and these activities could be related to their phenolic contents. This may also mean that secondary metabolites with antioxidant and antiradical activity are present in these extracts. (Nana 2017)

According to the phytochemical screening approach, It appears that the different extracts have practically the same constituents but the tannins are absent in the ethyl acetate extract and ethyl acetate from ethanol extract. The methanol and the ethyl acetate/ethanol extracts are the richest extracts containing the greatest quantities of secondary metabolites such as flavonoids, polyphenols and triterpenoids. All these compounds are well known for their biological activities. Previous investigation, some phytoconstituents of *S. ellipticum* leaf extract including five-triterpenoids, two-flavonoids, two-steroidal derivatives and one-phenolic compound were isolated from its leaf (Grewal et al. 2019). The presence of the flavonoids, phenolic and triterpenes compounds in the acetate and methanol extracts corroborates with previous research works carried out on *Sapium ellipticum* leaf, stem bark and root bark(Ighodaro et al. 2018, 2016;Mbayoet al. 2016; Ochwang'I et al. 2014;Nana et al. 2013). This result is also in agreement with those of some researchers who have found the same secondary metabolites in *S. ellipticum* leaf extract (Onukwuli et al. 2020) responsible for their use as antioxidant, antiplasmodial,

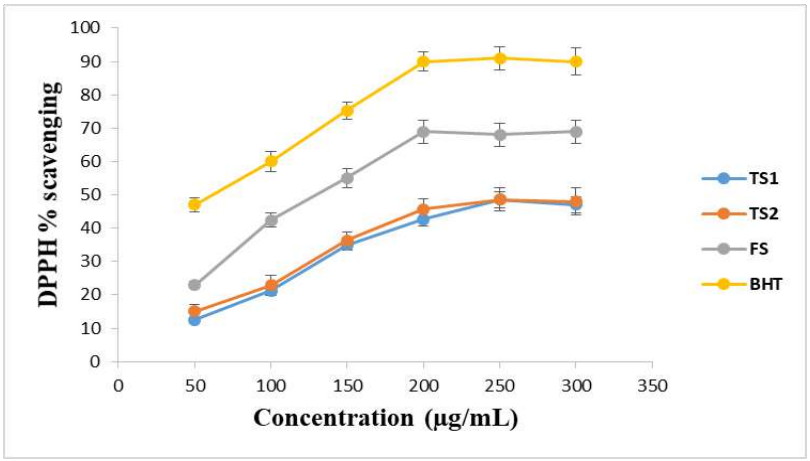
colorectal/oesophageal cancer, antidiabetic, and antidiarrheal. These activities could therefore explain and justify the different uses in folk medicine

3.3 Total phenolic, total flavonoid

The phenolic compounds are very useful because of their antioxidant capacity by inactivating lipid-free radical chains and avoiding hydroperoxide conversions into reactive oxyradicals. (Khorasani et al. 2015). These inactivation and reduction activities contribute to reducing or preventing the effects of oxidative stress on human health. Table 1 above shows the amounts of flavonoids and polyphenols contained in the extracts. The rich-flavonoid plants could manifest themselves as good sources of antioxidants that would assist in the enhancement of the overall antioxidant capacity of an organism and protection against lipid peroxidation. The total flavonoid content results were entirely synchronous with those of the total phenolic. It was successfully shown that samples with a high level of phenolic content also contain flavonoids in great amounts. The rich-flavonoid plant could be a satisfactory antioxidant source that would help become greater the overall antioxidant capacity of an organism and protect it against oxidative degradation of lipids.

3.4 DPPH radical scavenging activity

It emerges after the antioxidant evaluation activity using the DPPH radical scavenging ability of samples with different extraction solvents that, the ethyl acetate/EtOH extract of *S. ellipticum* root bark showed a reading of the lowest IC_{50} of DPPH radical scavenging (71.33%), while the highest IC_{50} belonged to the methanol fraction of *S. ellipticum* root bark (85.25%). Although the DPPH radical scavenging activities of the different extracts were less than those of the BHT taken as the reference (93.66%). This differential scavenging activities of the different extract against the DPPH system that has been observed could be explained by the presence of different classes of secondary metabolites in the extracts, more in the methanol fraction the most polar fraction because of the most polar solvent.More, free radical scavenging capacities, IC_{50} is generally described as the dose or concentration of a test material (extract plant, chemical, drug, etc.) that inhibits 50% of free radicals. Its values are useful in comparing the relative antioxidant capacity of molecules or plant extracts. The pure flavonoids (FS) and two pure triterpenoids (TS1, TS2) according to qualitative test isolated, were submitted to scavenging activities of 2, 2-Diphenyl-1-picrylhydrazyl radical and the antioxidant activity as a function of concentration was investigated.Fig.1 shows the results. According to this figure, the compound FS corresponding to a flavonoid exhibits the highest scavenging activity with an IC_{50} value of 140 µg. mL^{-1}compared to the two triterpenoids with an IC_{50} value of 275 µg. mL^{-1}. But the two values are less than the BHT IC_{50} value of 60 µg. mL^{-1}. As the natural phenolic compounds, FS is polyphenols are the major plant compounds with antioxidant activity and other several activities associated with healthy properties ascribed to their antioxidant activity and free radical scavenging abilities.(Wong et al. 2013). The two triterpenes show moderate scavenging activity. However, none of them was higher than BHT. The DPPH assay has been widely used for screening antioxidants due to its simplicity and reproducibility; more than that radical scavenging method is the main mechanism by which antioxidants act in foods. (Nana et al. 2021)

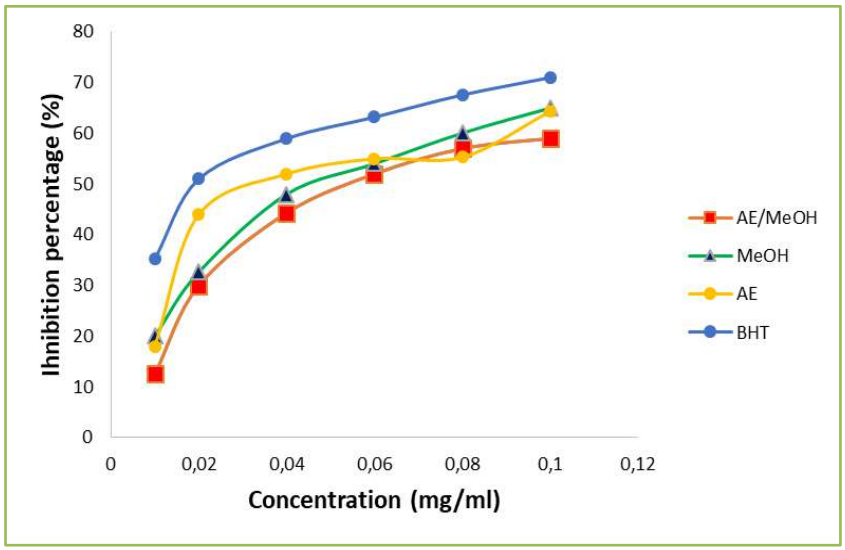


TS1: isolated compound 1, a triterpene from *S. ellipticum*; TS2: isolated compound 2, a triterpene from *S. ellipticum*; FS: isolated compound, a flavonoid from *S. ellipticum*

Fig.1 Scavenging activities of isolated compounds and BHT on DPPH radical

3.5 β-Carotene-Linoleic acid assay

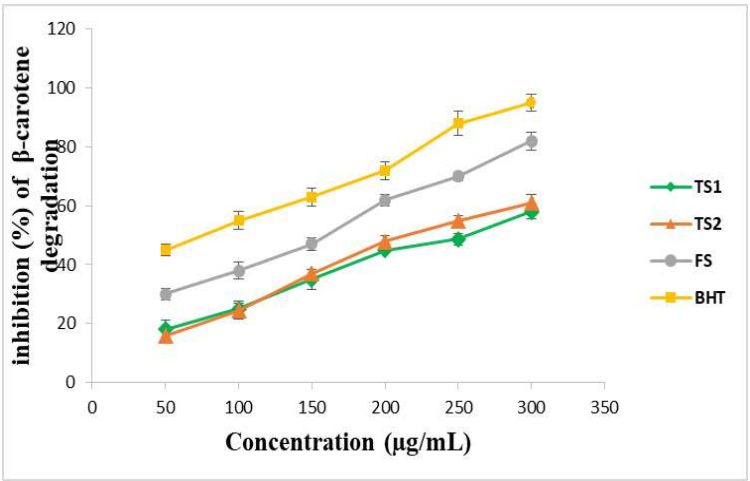
In this test, the antioxidant capacity of the three fractions is determined by measuring the inhibition percentage of the oxidative degradation of β-carotene by the oxidation products of linoleic acid (fig.2).The β-carotene bleaching method (coupled oxidation of β-carotene and linoleic acid) estimates the relative ability of antioxidant compounds in plant extracts to scavenge the radical of linoleic acid peroxide that oxidizes β-carotene in the emulsion phase. β-carotene in the absence of the antioxidant undergoes a rapid decolourization since the free linoleic acid radical attacks the β-carotene, which loses the double bonds and, consequently, its orange colour. According to this statement, *S. ellipticum* root bark extracts prevented discolouration of the couple β-carotene and linoleic acid (Nana et al. 2015)



MeOH: Methanol extract; AE: ethyl acetate extract; AE/EtOH: ethyl acetate extract from ethanol extract; BHT: butyl hydroxyl toluene

Fig.2 Inhibition percentage in the function of the concentration for *S. ellipticum.*

Figure3 below shows the values of IC_{50} for different isolated compounds obtained by using the curve of the variation of inhibition percentage in the function of concentration. These bioactive compounds are TS1, TS2 and FS3. The isolated compounds, exhibited strong antioxidant activities than crude extracts with IC_{50} values of 1.59 10^{-3}, .2.6 10^{-3} and 7.9 10^{-4} mg. mL^{-1} respectively compared to crude ethyl acetate extract with IC_{50} values of 2. 10^{-2}mg. mL^{-1}, crude EtOAc/EtOH extract with IC_{50} values of 5.4.10^{-2}mg. mL^{-1} and crude MeOH extract with IC_{50} value of 11.02.10^{-3}mg. mL^{-1} but less antioxidant capacity than BHT with IC_{50}value of 3.36 µg.ml^{-1} taking as control. These calculation values are in agreement with the deduced values below in Fig 3. These β-carotene-linoleic acid test values as DPPH test values are following the fact that the antioxidant and antiradical capacity of the obtained extracts and molecules isolated from *S. ellipticum* is directly correlated to the concentration of polyphenols; flavonoids taken as polyphenols obey to this statement compared to terpenoids.



TS1: isolated compound 1, a triterpene from *S. ellipticum*; TS2: isolated compound 2, a triterpene from *S. ellipticum*; FS: isolated compound, a flavonoid from *S. ellipticum*

Fig.3 Inhibition percentage in the function of the concentration for *Sapiumellipticum* by using β carotene and linoleic acid system.

3.6 Correlation between antioxidant activities and phytochemical contents

By this analysis of the correlation between the biological activity and the family of the compounds, it appears that for phytochemicalcontentswithIC_{50} values of radical scavenging activity and antioxidant ability of the ethyl acetate extract of *S. ellipticum* root bark and its various solvent fractions, the phenolic and flavonoid contents had exhibited excellent link with DPPH andoxidative degradation of β carotene of *Sapium* ethyl acetate extract samples (Table3). These results are close to those found by Erkan(2008) and Khorasani (2015) who reported in their previous works on the antioxidant activities of rosemary (*Rosmarinus officinalis* L.) extract, black seed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol on antioxidant activity and total phenolic and flavonoid content of various solvent extracts from *in vivo* and *in vitro* grown *Trifolium pratense* L. (Red Clover)

Table 3 Correlation between the antioxidant activity and total phenolic and total flavonoid of *S. ellipticum* root bark extract.

Assays	CorrelationR^2(%)	
	Total flavonoid	Total phenolic
DPPH radical scavenging activity	90.3**	87.8**
Oxidative degradation of β carotene	85.8*	91.3**

**Correlation is significant at the 0.01 level

3.7 Isolatedpure compounds

One pure flavonoid (FS1) and two pure triterpenoids (TS1, TS2) according to a qualitative test were isolated, close to a powder, they came in the form of very thin yellow granules for flavonoids and reddish-orange granules for triterpene. The masses of (10 mg) and (6 mg); (8 mg); were obtained and labelled FS1, TS1 and TS2 respectively. FS1 is a flavonoid, and some are endowed with vasculoprotective, venotonic, antiviral, antithrombotic, antimicrobial, anticancer, and anti-inflammatory properties. According to the chemical structure of the flavonoids, not yet knowing the structure of the isolated flavonoids, in general, the ortho-dihydroxy structure on B ring confers stability to the flavonoxy radical and participates in the delocalization of electrons thus increasing the antiradical potential which is altered by the presence of methoxy group on B ring. More than that, the conjugated double bond C2-C3 with the 4-oxo group on the C ring increase the radical scavenger capacity of flavonoids. In addition, the presence of the3-OH group in combination with the C2-C3 double bond also increases the radical scavenger capacity of the

flavonoids (Balasundram 2006). The two triterpenes labelled TS1 and TS2 are also isolated from *S.ellipticum* root bark. This work is in agreement with a previous study that led to the obtention, from the active fractions of *the ethanolic extract of S. ellipticum* leaves, of three triterpenoids named lup-20(29)-en-3-one, β-amyrin and acetyl aleuritolic acid and one steroid β-sitosterol (Ighodaro et al. 2018). The potential biological activities of triterpenoids include anti-inflammatory, anticarcinogenic, antidiabetic, hepatoprotective, antimicrobial antimycotic, analgesic, immunomodulatory, and cardiotonic activities (Wang et al. 2021)

4. Conclusion

Based on the different biological activities studied in this present work, flavonoids and triterpenes are two groups of secondary metabolites with many biological activities present in *S. ellipticum* root bark. Two terpenoids and one flavonoid were isolated from *Sapium ellipticum* (Hochst) Pax root bark labelled TS1, TS2 and FS3 exhibited strong antioxidant activities with IC_{50} values of 1.59 10^{-3}, .2.6 10^{-3} and 7.9 10^{-4} mg/mL. In addition, terpenoids are known to induce apoptosis inducers while flavonoids are powerful antioxidants that eliminate free radicals released by oxidative stress. Other studies will be carried out to elucidate the molecules structures and secondarily to isolate new compounds from the remaining extracts namely methanol and ethanol extracts. *Sapium ellipticum* extracts have proven good antioxidant activities thus justifying their use in the prophylaxis of cardiovascular diseases, and cancers and this lends some credence to its widespread traditional use by the North Cameroon local population.

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