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Qualitative characterization of phenolic profile of the fruits and leaves of *Solanum torvum* Swart naturally growing in Côte d'Ivoire

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Abstract

The polyphenol phytochemical constituents have a positive impact on health and in the prevention of various diseases. The current study aimed at qualititative phytochemical analysis of *Solanum torvum* from Côte d'Ivoire, in order to knowledge phenolic potential of plant for futurs reseachs. The leaves and fruits of *Solanum torvum* were collected in Daloa province of Côte d'Ivoire, dried, ground to powder and extracted using differents solvents. The dry extract purified was dissolved in methanol and filtered through a Millipore membrane with 0.22µm porosity. Then, each of extracts was further subjected to TLC and HPLC. Total phenolic and flavonoid contents were evaluated using Folin-Ciocalteu method and aluminium trichloride test, respectively. The results revealed that leaves extracts accumulated significantly higher phenolic content 86.07±1.49mg GAE/g DW and flavonoid content 2.51±0.14mg GAE/g DW than extracts fruits. The chromatography analyse showed eigteen compounds in fruits fraction and twenty compounds in leaves fraction. Chromatography identification conducted on ethyl acetate fraction revealed the presence of phenolics acid, flavonoids and coumaric acid. Most of identified compounds are already were isolated by previous phytochemical investigations on *Solanum* except arbutin, tannic acid and p-coumaric acid. These major active compounds have various biological activities and play a known role in maintaining good health. The results show *Solanum* from Côte d'Ivoire have the same abilities as other species in the world. The knowledge of phenolic profile of *Solanum torvum* from Côte d'Ivoire are very important for their authentication, conservation and utilization of biological resources.

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Introduction

Solanum torvum Swartz commonly known as Turkey berry is a small shrub is widely distributed in tropical and subtropical areas such in Côte d'Ivoire. It belongs to the family Solanaceae. The genus *Solanum* consist of more than 1500 species. This species has several synonymies such as *Solanum ferrugineum* Jacq, *Solanum largiflorum* C. White, *Solanum fisifolium* Ortega accorting to different flora (Colmenares *et al.*, 2013). Previous phytochimical investigations on *Solanum torvum* have led to isolation of steriols, steriol glycoside, triterpenes isoflavonoid sulfate and steriol glycosides (Yousaf *et al.*, 2013), and saponines and flavones (Yuan-Yuan *et al.*, 2011). The authors (Arif and Fareed, 2011) indicate that *Solanum torvum* as an excellent source of natural antioxidants. The nutritional and mineral composition of *Solanum torvum* fruits have reported (Osei Akoto *et al.*, 2015). The fruits are used as a vegetable and the whole plant is used in the traditional medicine for the treatment of wide panel of diseases. On the basis of the richness in various chemical compounds of this species and its biodiversity, studies of *Solanum* from Côte d'Ivoire are required to knowledge this its plant and to encourage its consumption because considered toxic by the population. For that, investigate the polyphenol composition of plant of *Solanum torvum* from Côte d'Ivoire was undertaken. Indeed, the polyphenol have a positive impact on health and in the prevention of various diseases. in addition to phenolic compounds and flavonoids, have a role as antioxidant and detoxifying agents. The intake of dietary antioxidant phytochemicals leads to protection against noncommunicable diseases such as cancer, cardiovascular diseases and cataract (Kaunda *et al.*, 2019). During the last few decades there had been an increasing interest in the study of medicinal plants in different parts of the world to authentication, the conservation and utilization of biological resources to plant studied (Lev, 2006). For this purpose, the current study aimed at qualititative phytochemical analysis of *Solanum torvum* from Côte d'Ivoire, in order to knowledge phenolic potential of this plant for futurs reseachs. the extracts of leaves and fruits of S. torvum collected in Daloa province of Côte d'Ivoire were further subjected to Thin Layer

Chromatography (TLC) and high-performance liquid chromatography (HPLC) coupled of HPLC-MS.

Materials and methods

Plant material

Fruits and leave of *Solanum torvum Swartz* were collected during Mai 2017, in Daloa province of Côte d'Ivoire. Collected plant material was left at room temperature for 15 days, dried in shaded and well-ventilated place, then kept refrigerated in glass containers before further processing.

Sample preparation and extraction

The sample of fruits and leaves dried were crushed with a mixer. Organ extracts were obtained by refluxing of 50g of dry organ powder in 60% acetone (v/v) at temperature 25°C, using two cycles of 2h with blender. The extracts were filtered through filter paper and concentrated with a vacuum rotary evaporator HAHNVAPOR HS-2005 V-N to obtain a crude water extract. The water extract was resuspended in 50% (v/v) petroleum ether and then partitioned sequentially with petroleum ether, ethyl acetate and water. Petroleum ether fraction (F_1), ethyl acetate fraction (F_2) and water fraction (F_0) were collected separately and concentrated using a vacuum rotary evaporator to remove the solvent. All fractions were lyophilized and stored at - 20°C until analysis.

The yield of evaporated and lyophilized dried extracts percentage was calculated as following:

% Yield = ($DW_{extract}$ /DW_{organe}) X 100

$DW_{extract}$: dry weight of extract after lyophilisation,

DW_{organ}: dry weight of organ sample.

A fine dried powdered each sample (2mg) were dissolved in 30% methanol and purified. The methanol/ water extract (1ml) was subjected to Visiprep system on silice C18 previously regenerated with 2ml of methanol (100% and 50%) and water. The methanol extract was eluted by 2 x 2ml of methanol 90%. The fraction eluted was evaporated to dryness. The fraction purified and concentrated was dissolved in 1ml methanol and diluted with an equal

volume with bidistilled water was filtered through a 0.22µm Millipore filter. The filtrate obtained was used for all analyzes.

Total phenolic content

Total phenolic content was determined according to the Folin-Ciocalteu method (Gorge *et al.*, 2005). 0.1ml of the filtrate were added 0.9ml distilled water and 0,5ml of Folin –Ciocalteu reagent 1N (Sigma Chemical Co., Etats-Unis) and 1,5ml of sodium carbonate solution (17%, p/v). The mixture was incubated at ambient temperature. After 30 min, the absorbance was measured at 760nm. The total phenolic content was determined using the standard gallic acid (Sigma Chemical Co., Etats-Unis) calibration curve and the results were expressed asmg gallic acid equivalents per gram dry weight (mg GAE/g DW). All samples were analysed in triplicate.

Total flavonoids content

The total flavonoids content of each sample was determined following Padmaia *et al.* (2011) modified method. 100µl of each filtrate were mixed with 1.49ml distilled water, 300µl of solution of 5% sodium nitrite. After 5 min, 300 µl of solution 10% aluminium trichloride solution were added. After 6 min to stand, 0,2ml of solution hydroxyde de sodium (1M) was and distilled water were added to mixture bring the final volume to 5ml. The mixture was homogenized and the absorbance was measured at 510nm against a blank content the methanol. A standard curve was prepared using catechin in methanol under the same conditions. The total flavonoids content was expressed as milligrams of catechin equivalents per gram of dry weight (mg CE/g DW).

Thin Layer Chromatography screening

The thin layer chromatography (TLC) analysis was use for polyphenol screening. The filtrate (100µl) was analyzed over silica gel plates (Merck) by comparison with standards following: rutin, quercetin, kaempferol, caffeic acid, gallic acid, ferulic acid and chlorogenic. Sample (10μL) was spotted on precoated Silica gel plates. The plate was spotted using CAMAG ATS3 apparatus then the plate was developed in with the different solvent system chloroform–methanol-glacial acetic acid (80:20:3, v/v/v) as mobile phase. After drying, the plates were sprayed with vanillin-sulfuric acid reagent and heating at 100°C for 5 min. After heating, chromatograms were observed under UV light at 254 nm and 365nm. The retention factor (Rf) was calculated using the following equation:

Rf = Distance move by the substance (cm) / Distance move by the solvent (cm)

Phenolic compounds by HPLC analysis

Leaf and fruit water and ethyl acetate fraction were characterized by high-performance liquid chromatography (HPLC) analysis. This analysis was conducted according to the method described (Yapo *et al.*, 2011). Analyses were performed on a HPLC Agilent, model-LC 1100 series, equipped with a degasser, an autosampler automatic injector, a high pressure pump and a UV/Visible detector at multiple wavelengths wave, and running on Windows XP Workstation. A reversed-phase C18 column (Prontosil, 250 x 8.0mm, 5µm, Bischoff) using a binary gradient eluent (solvent A : 0.025% trifluoroacetic acid in water ; solvent B : acetonitrile pur) was employed at a flow rate of 0.6ml/ min. The elution conditions were optimized with program follows 15–50% B (0–60 min), 50–100% B (60–61 min), 100% B (61–70 min). The column was then washed 100% B, returned to the initial conditions and re-equilibrated for 6 min, resulting in a total run time of 40 min. An aliquot (20µL) of each filtrate were injected through the autosampler for each run and elution profiles were monitored at 284nm.

Phenolic compounds identification was achieved by the absorbance recorded in the chromatograms relative to external standard. A range of forty standards was used. A monitoring wavelength of 284nm was also used for qualitative analysis of phenolic compounds in the extract. The identity of the compounds corresponding to peak are based on their retention times, comparing with spectra standards. HPLC-MS analyse was developed for separation and confirmation of preliminary identification with reference standards. Analyse were performed on an Agilent 1200 infinty series HPLC-MS system (Agilent technologies Inc.CA,

Germany) with Phenomenex Kinetex XB-C18 column, 2.1 x 100mm, 1.7µm particle size and 100-A° pore size (Phenomenex, CA, Germany).

All other chemicals and solvents were of HPLC-grade purity from Sigma-Aldrich (Steinheim, Germany). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA) before use.

Statistical analysis

Experiments were carried out in triplicate. Experimental data were subjected to analysis of variance (ANOVA) using statistical software (release 7.1). Differences between the means of data obtained were compared using Newman-Keuls test. Differences at $P < 0.05$ were considered as significant.

Results

Extraction yields, total phenolic content and total flavonoid content of Solanum torvum leaf and fruit

The extraction of *Solanum torvum* leaves and fruits gave three fractions each. The results of using different solvents for the extraction of phenolic compounds are given in Table 1. The extraction yield varied from 1.46 to 7.57% for fruits extraction. Among all the fractions, the water fraction (F0) obtained the highest extraction yield (7.57%) while Petroleum ether fraction (F1) yielded the lowest (1.46%). The yield of all fractions are classified in the following order F0> F2> F1.

Table 1. Extraction yields and polyphenol content in leaf and fruit fraction of *Solanum torvum*.

Samples	Extraction yields	Total phenol	Total flavonoid
	%	mg GAE/g DW	mg CE/g DW
LeF0	7,57 ±0.14a	25.72±0.02c	1.75±0.01a
LeF1	0,46 ±0.25c	0.34±0.27d	0.15±0.05b
LeF2	2,03± 0.34	86.07±1.49a	2.51±0.14a
FrF0	11,26±0.19a	1.56±0.01d	0.23±0,02b
FrF1	3,36±0.07b	0.56±0.03d	0.03±0.01b
FrF2	3,49±0.31b	65.45±1.06b	1.64±1,09a

F0 : water fraction, F1 : petroleum ether fraction, F2 : Acetate ethyl fraction, Le : leaf, Fr : Fruit ; values are means of triplicate determination ± standard deviation (SD) ; in a line and a column, values followed of a same letter are not statistically different (test of Newman-Keuls at 5%).

The results of total phenolic content of the different fraction of leaves and fruits studied are giving in the table 1. The amount of total phenolic varied from 0.56±0.001 to 86.1±1.49mg GAE/g of dry weight. The quantitative analysis of total phenolic content showed that acetate ethyl fraction indicates a high significant phenolic content with 86.1±1.49mg GAE/g of dry weight for leaves and 25.45±1.09mg GAE/g of dry weight for fruits. For the total flavonoid content, the same tendency was observed. And the total flavonoid content is significantly higher in leaves fraction than the fruit fraction (table 1).

TLC screening and compounds identification

The results of TLC approach were followed to identify the phenolic compound in fraction of *Solanum torvum* (Table 2). The Rf values and color spots of *Solanum* fraction were compared which the standards. Qualitative TLC analysis revealed the presence of high levels of some secondary metabolites in the *Solanum torvum*. The differents extracts of *Solanum torvum* showed the presence of flavonoids, phenolic acids compounds and some unidentified compounds are present in all fraction.

Chromatographic profils of extracts and compound identification

The analyse of HPLC chromatograms obtained (Fig. 1) show the presence of several phenolic compounds in water and ethyl acetate fractions of *Solanum torvum* leaf and fruits. Some phenolic compounds were identified by comparing their retention time with similar compounds that have been previously studied under similar conditions. The examination of chromatogram showed 18 peaks for fruits fraction and 20 peaks for leaves fraction were detected. Compounds identification yielded several matches. For fruits fraction, the identities of 8 phenolics compounds were confirmed within the water fraction and 18 compounds within the ethyl acetate fraction (Table 3). For leaves fraction, 9 compounds were identified within the water fraction and 17 compounds within ethyl acetate fraction (Table 3). In total, 22 different compouds were detected in the fraction. The phenolic profiles obtained show the presence of tannic acid, the phenolic acids, flavonoids

acid, coumaric acid and most compounds identified by previous phytochemical investigations on *Solanum* except tannic acid and p-coumaric acid.

Table 2. TLC Screening of leaves and fruits extract of *Solanum torvum*, Rf values and color of spot. Revelation: Vanillin-H_2SO_4.

Fraction	Leaves				Fruits			
	Rf	UV254	UV365	Identified compounds	Rf	UV256	UV365	Identified compounds
Water	00	-	-	-	0.00	grey	Fluores	-
	0.13	green	pink	-	0.38	beige	purple	Rutin
	0.37	grey	purple	Rutin	0.54	Grey	Fluores	Chlorogenic
	0.41	brown	fluore	Ferulic ac.	0.58	green^{J}	Orange	Kaempferol
	0.48	blue	Blue^{F}	Galic acid.	0.62	green	violet	Cafeic ac.
	0.53	green	fluoresc	Chlorogenic	0.77	Beige	pink	Quertin
Petroleum ether	0.13	green	pink	-	00	Grey	fluores	-
	0.37	green	purple	Rutin	0.39	beige	pink	Rutin
Ethyl acetate	0.13	green	purple	-	0.37	beige	pink	Rutin
	0.37	green^{C}	purple	Rutin	0.42	green^{F}	Fluores	Ferulic ac
	0.48	green	Blue^{F}	Galic acid	0.48	yellow	Blue^{f}	Galic acid
	0.52	Blue	purple	-	0.54	Beige	fluores	Chlorogenic
	0.57	green	orange	Kaempferol	0.59	green^{J}	purple^{O}	Kaempferol
	0.64	greenj	violet	Cafeic acid	0.62	green	violet	Cafeic ac.
	0.76	beige	pink	Quertin	0.79	Beige	pink	Quertin
Standards	0.37	green	purple	Rutin	-	-	-	-
	0.77	beige	pink	Quercetin	-	-	-	-
	0.58	green^{J}	orange	Kaempferol	-	-	-	-
	0.61	green^{F}	violet	Cafeic acid	-	-	-	-
	0.49	yellow	blue^{F}	Gallic acid	-	-	-	-
	0.42	blue	fluores	Ferulic acid	-	-	-	-
	0.54	beige	fluores	Chlorogenic	-	-	-	-

Mobile phase: chloroform–methanol-glacial acetic acid (80:20:3, v/v/v)

^{C}: pale; ^{F}: dark; ^{J}: yellomish; ^{O}: orange; fluores=fluorescent

Table 3. *Solanum torvum* Swart from Côte d'Ivoire phenolic compounds idenfied by HPLC coupled of HPLC-MS.

N° Peaks FrFo	N° Peaks FrF_2	Retention time (mins)	Identified compounds	N° Peaks LeFo	N° Peaks LeF2	Retention time (mins)	Identified compounds
1	1	2.680	Arbutin	1	1	2.680	Arbutin
2	2	3.687	Tannic acid	2	2	3.704	Tannic acid
3	3	4.332	3,4dihydroxybenzoic acid		3	4.385	3,4dihydroxybenzoic acid
	4	4.833	Methyl gallate	3		4.518	Gallic acid
4	5	5.313	Protocatechic aci.		4	5.972	Catechin acid
5	6	5.934	Catechin acid	4	5	6.689	Chlorogenic acid
	7	6.612	Chlorogenic acid		6	7.392	Rutine
6	8	6.980	syringaldehyde	5	7	8.043	Caffeoylquinic ac
	9	7.585	Rutine	6	8	8.341	Cafeic acid
7	10	8.332	Cafeic acid	7	9	8.842	Epicatechin acid
8	11	10.049	P-coumaric acid		10	10.102	P-coumaric acid
	12	10.487	Myricetin	8	11	10.676	Myricetin
	13	11.604	Quercitrin acid		12	11.493	Quercitrin acid
	14	12.661	Isoquercitrin		13	12.763	Isoquercitrin
	15	14.209	Isoferulic acid	9	14	24.611	Ferulic acid

16	15.073	Taxifolin	15	27.735	Quercetin acid
17	16.992	Myricitrin	16	29.747	Kaempferol
18	24.838	Ferulic acid	17	30.468	Kaempferol glucoside

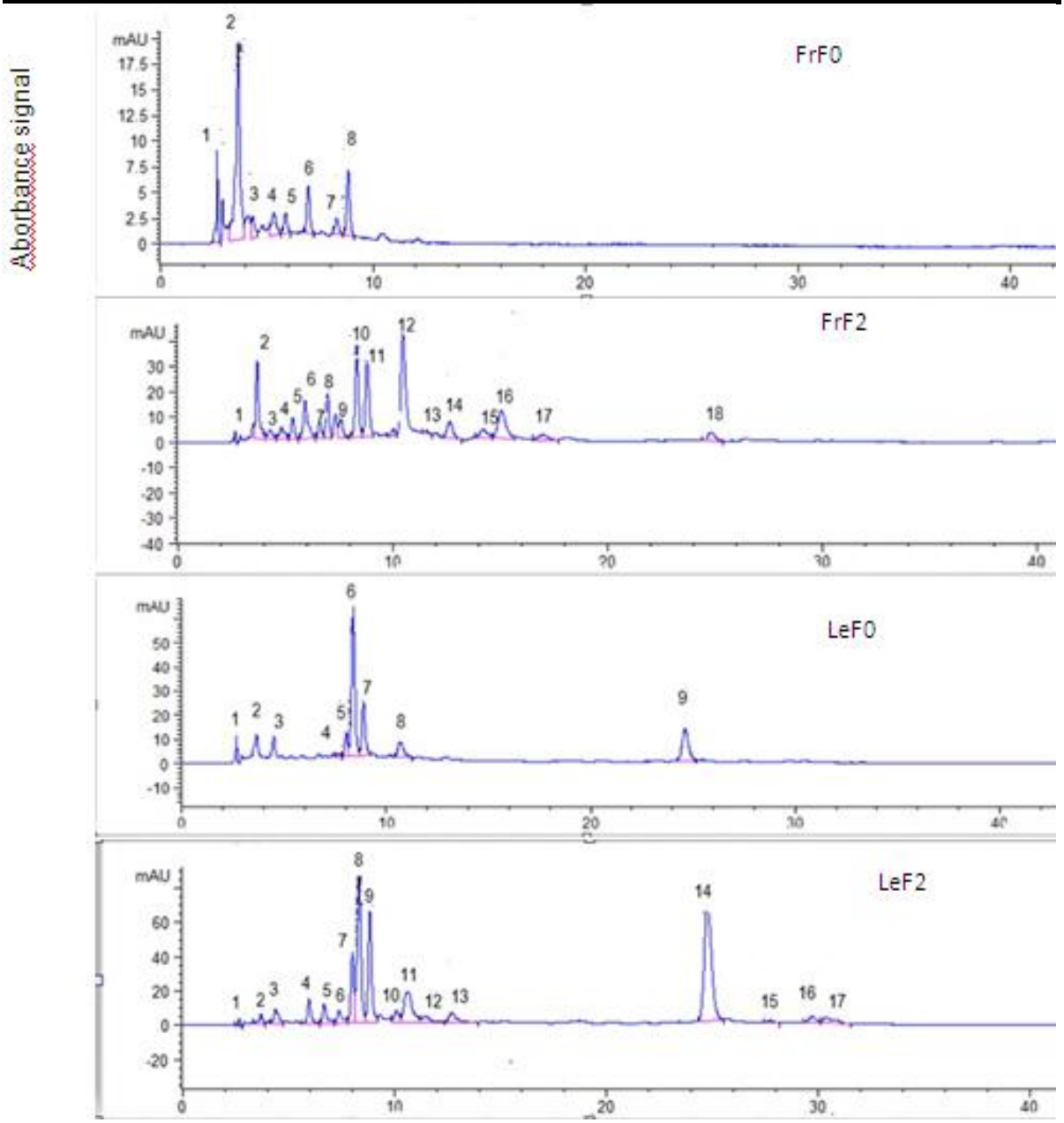

Fig. 1. HPLC chromatograms of phenolic compounds detected in extract of the fruits and leaves using 284 nm absorbance detection**.** Numbered peaks represent identified compounds as reported in Table 3. FrF0, fruit water fraction ; FrF2, fruit ethyl acetate ; LeF0, leaf water fraction ; LeF2, leaf ethyl acetate.

Discussion

In the present work, the result showed that whatever the organ used, the high extraction yield was given by water solvent. This high extraction yield by water is probably due to high solubility of major components of *Solanum torvum* in water. However, the sample of fruit have the yield highest than the leaves. This observation show that the extraction yields depend on both the organ of the plant and the extraction solvent. This observation can be explained by the fact that water is a highly polar solvent known to extract a wide range of molecules. The variation in the yields of extracts could be attributed to the difference in solvent polarities used which also plays a key role in increasing the solubility of phytochemical compounds (Naima *et al.*, 2015). Moreover, our results seem lowest compared

to previous studies. Therefore, our extraction method used certainly played a role in the decrease the extractive content productive potential of plant. Our results confirm that choices of solvent and extraction method play important roles on maximizing extract yield (Manisha Gahlot *et al.*, 2018).

On the other hand, the total phenolic content was increasing by creasing order of the polarity of solvents enter ethyl acetate and water. For the organs, the leaves have the highest levels of phenolic compounds content than the fruit. The unequal distribution of polyphenols in different organs of the same plant has been reported by several authors (El-Hacl *et al.*, 2012). For those authors, this unequal content is due to several factors such as extraction method, solvent, number of extraction steps, expression of the results, (Luthria, 2008, Rodriguez-Rojo *et al.*, 2012). In addition, the results obtained are not in agreement with the previous findings. The values obtained with ethyl acetate fraction were four times as high as the ones reported by Asmah *et al.* (2007). In addition, all results obtained are contrary to those results obtained by Arif and Fareed (2011) with S. torvum's samples collected in India area and those gave by Abdulaziz R. *et al.* (2016) with S. torvum collected in Malaysia. Also, the phenolic profiles obtained show that the majority of phenolic compounds identified correspond to those already isolated by previous phytochemical investigations on Solanum except tannic acid and p-coumaric. The variability observed between the different results according the study areas might be due to solvent used for extraction, environmental factors, fresh or dry samples, and the standard compounds used (Abdulaziz R. *et al.*, 2016). According Kuti (2005), these results might be due to factors physiological, genetic and environment factors of plants. The differences observed between our results and previous studies suggest that there is a correlation between the secondary metabolites content in S. torvum plant and origin geographically area factors of the plant growth. Indeed, many environmental factors can act singly or interact on medicinal plants to affect the productivity of its secondary metabolites (Gololo *et al.*, 2018). Knowing that the photosynthesis is an important precursor in all the mechanisms of synthesis of molecules within the plant. Photosynthesis is itself influenced by many genetic factors such as age and leaf morphology and environmental factors like light, the availability of carbon dioxide, temperature, soil moisture, nutrients and canopy structure (Hopkins, 2003). So, we can say that all differences observed would be due to geographical facteurs. In addition, the presence of coumarines, flavonoids and phenolic acids in the extract indicate a biological activity of the extracts. That was confirm by bacteriogical test (O C Okou *et al.*, 2019).

Conclusion

The present work conform clearly the presence of bioactive compound in *Solanum torvum* come from Côte d'Ivoire. So, it has possesses a wide spectrum of pharmacological activities as all Solanum of the world. The knowledge of this plant would help promote it in Côte d'Ivoire. The aim is to encourage the population to use the plant as a food ingredient to maintain their health and as biological resource for other researchs.

Conflict of interest

We declare that we have no conflict of interest.

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