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Research Article

UV-Visible spectrophotometric detection of *Solanum aethiopicum*hexanic and n-butanolic excerpts and their anticancer activity monitored by flow cytometry and microscopy

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ABSTRACT

UV-Visible Spectrophotometry (UV-Vis) is one of the most used analytical technics because of its accuracy, however, it application in phytochemicals and extracts characterization still a huge challenge even for the choice of appropriate wavelength at HPLC detector. TLC screening of *Solanum aethiopicum*L.(Solanaceae) hexanic and n-butanolic excerpts respectively F1, F4 has been reported but these extracts have never been characterized by UV-Vis. Likewise the study of their potential effect against cancer cells using microscopy and flow cytometry has never been performed. The aim of this work was firstly to characterize F1 and F4 by UV-Vis, find out the possible embedded phytochemicals and secondly to assess their anticancer potential. The findings show that F1 has 33 major phytocompounds detected from 176-254 nm while F4 contained 45 detected between λ 176-679 nm and mostly identified as sterols, terpenes, phenolic acids and flavonoids which may be responsible oftheiranti-proliferative effects againstJurkat cells (Leukemia cancer).

Keywords:*Spectrophotometry, phytochemicals, Solanum aethiopicum excerpts, anticancer*

INTRODUCTION:

Thin-layer chromatography (TLC) is one of the most used technique for characterizing the different classes of plant secondary metabolites or phytocompounds (PCD) by using fixed wavelengths and different reagents. In addition to TLC, Ultraviolet (UV) and Visible spectrophotometry seem to be a fast method for accurately characterize PCD based on their wavelengths of light absorption, in this case a full scanning is performed and the different peaks corresponding to light absorption of specific compounds need to be identify, the principle is based on Beer–Lambert law which link absorbance to sample concentration[1]. Even in a mixture, the wavelength *λ* of a compound is specific and can be used as identification parameter[2, 3]. It appears to be time consuming when identifying the phytochemicals contained in plant extracts by HPLC and the choice of appropriated detector's wavelength can be a real issue as extract contains different type of PCD having different maximum light absorption. In this study, phytocompounds contained in *Solanum aethiopicum* crude excerptsF1 and F4 have been analyzed by UV-Visible spectrophotometry and the detected wavelengths of PCD have been attributed to those of previous studies. According to the findings, it is likely that PCD can be accurately analysed using the appropriate wavelengths for specific classes of secondary metabolites.

MATERIALS AND METHODS

Plant material and extractions

The plant material, extraction procedures of F1 and F4 have been previously reported[4,5].

UV-visible spectrophotometry analysis

F1 (331 mg/ml) and F4 (331 mg/ml) in phosphate buffered saline (PBS) solution[6]were analyzed after the blank using a scan spectrophotometer UV-visible type Varian Cary 50 BIOPty Ltd. ACN 004 559 540 from Australia; the scan range set between 200-800 nm and 200 - 400 nm. Thereafter, 1 µl of each extract solution was used for Nanodrop (scan between 176 - 659 nm) UV-Vis

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analysisusingNanodrop 1000 Spectrophotometer from thermo scientific. The maximum wavelength of each peak was then attributed to corresponding classes of phytocompounds based on previous findings.

Flow cytometry and microscopic monitoring

The procedure of Jurkat cells culturing is known[5];0.5x10[6] viable Jurkat cells were used in 96 wells plates round bottom (Sarstedtinc. Newton, USA). After the treatment of the cells using 0.3, 33 µg/ml and0.5, 7.5, 12.5 and 25 mg/ml of F1 (RPMI-1640), all the cells including the controls (untreated) were incubated under 37°C for 24 and 48 hours[5]. After 24 h of treatment, the cells treated with 0.3, 33 µg/ml of F1 and 15, 25 mg/ml of F4 were analyzed by flow cytometry (BD Accuri C6 de BD Biosciences (BD Accuri™C6 flow cytometer instrument manual 7820018) according to the user manual to estimatethe percentage of viable cells in the set gate, a subsequent decrease in the percentage of viable cellsis link to the influence of the extract on the cells. Thereafter at 48 h, the remaining treated cells were pictured using an optical Microscope CKX41SF, Olympus Optical Co. LTD, Tokyo, Japan in order to have a closer look on the effect ofF1 and F4 over Jurkat cancer cells.

DATA ANALYSIS

Data analyses were performed three times to insure reproducibility of identified peaks, when applicable, the software Origin 6.1 (OriginLab) and Matlab 7.0.4 (MathWorks) were used. Cytotoxicity experiments were also triple replicated and the treated cells with extracts were compared to the control (untreated) cells and the viability of the cells assessed.

RESULTS

UV-visible spectrophotometry characterization F1 and F4

In comparison to TLC where the characterization and identification of phytochemicals is based of R_f(Retention factor) and the change in coloration upon different reagents, in UV-Vis spectrophotometry, PCDcan be directly detected based on their wavelength of light absorption. Figure 1 shows the spectra of F1 and F4, scanned between 200 and 800 nm, the presence of a particular compound at a particular wavelength is indicated by the shift in the shape of the spectrum. From the crude extract chromatogram (Figure 5), the richness of F1 and F4 in several of phytochemicals seems obvious.

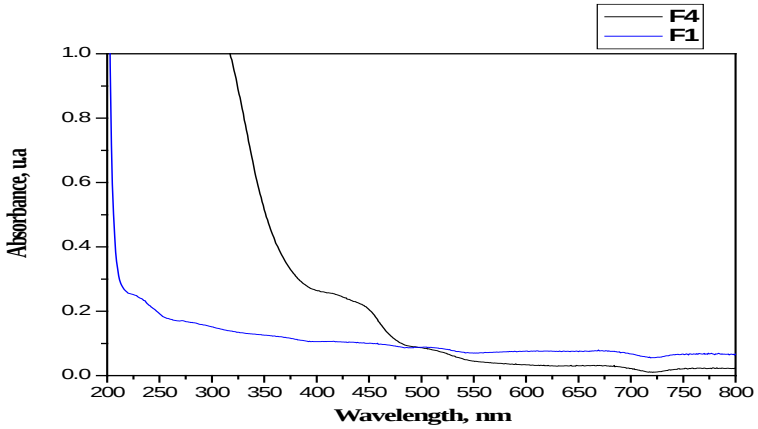

Figure 1: UV-Vis spectra of F1 and F4; absorbance vs wavelength of absorption λ (nm)

[F1]= [F4] =331 mg/ml in PBS for all UV-Vis analyses, Scanned between 176-800 nm

These analyses show that, the absorbance, peaks amplitude and the sample concentration are in the same order and interconnected; the higher the concentration, high absorbance is expected. At absorbance ≤ 1, it is clear that F1 and F4 contain some PCD absorbing both in UV and Visible and the spectra show that PCD of F1 absorb at wavelengths< 400 nm and the majorityat wavelengths< 250 nm. F4 richness in phytochemicals is detailed in the following Figure 2 with revealed wavelengths of some of the majorphytocompounds.

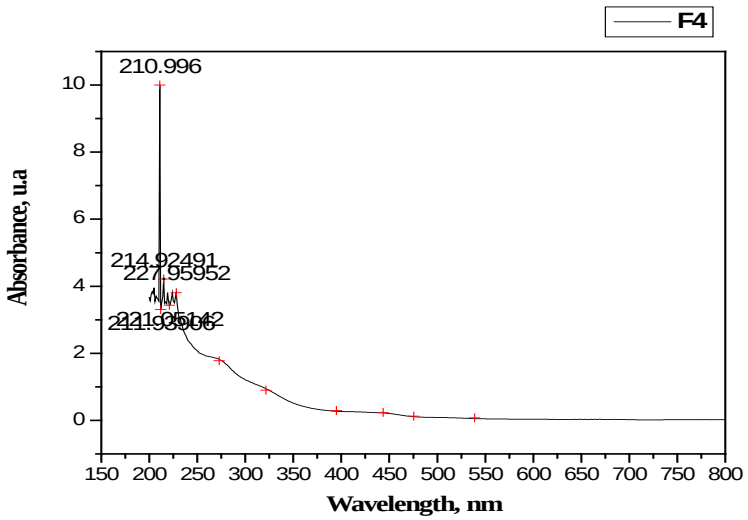

Figure 2: UV-Vis spectrum of F4; absorbance vs wavelength of absorption λ (nm)

Interestingly, it is visible that the PCD of F4 with higher concentrations in the extract are localized in UV, moreover, figure 2 displaystheir corresponding wavelengths. For more accurate identification of these natural products, more specific analysis were carried out using both Nanodropspectrophotometry (scan range, λ176-800 nm) and the scan spectrophotometer for UV analysis scanned between 200 to 400 nm. Figure 3-4 describe the results obtained from UV analyses of F1 and F4.

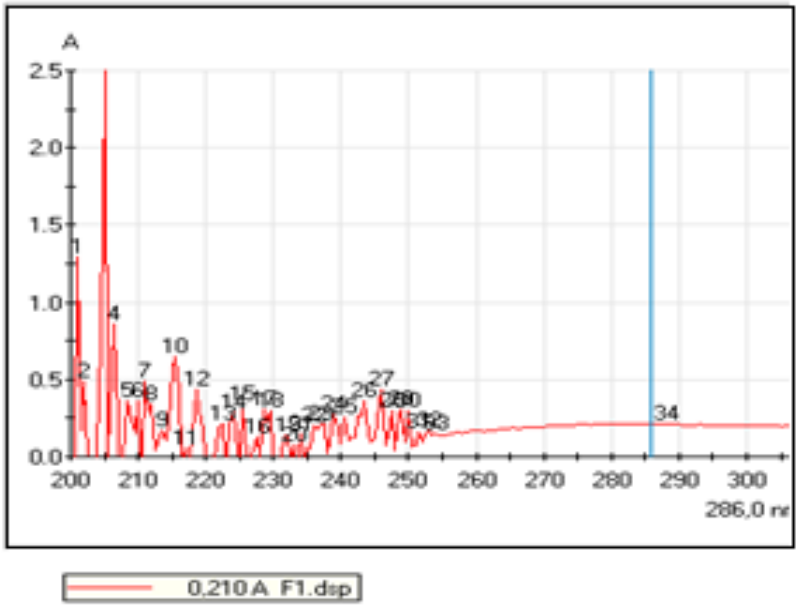

Figure 3: UV spectrum of F1; absorbance vs wavelength of absorptionλ (nm)

Scanned between 200-400 nm

Figure 3-4 confirm previous observations of figure 1-2; several compounds of F1 and F4 are detected in UV, F1 contains at least 33 peaks characterizing the presence of PCDand the major products showed by peaks 1, 2, 3, 4, 7, 8, 10 and 12 with corresponding wavelengths 202, 206, 208, 212, 213, 215 and 220 nm. However, Figure 4 highlightssomePCDcontained in F4, the richness of F1 and F4 in phytochemicals was confirmed[6] (Figure 5).

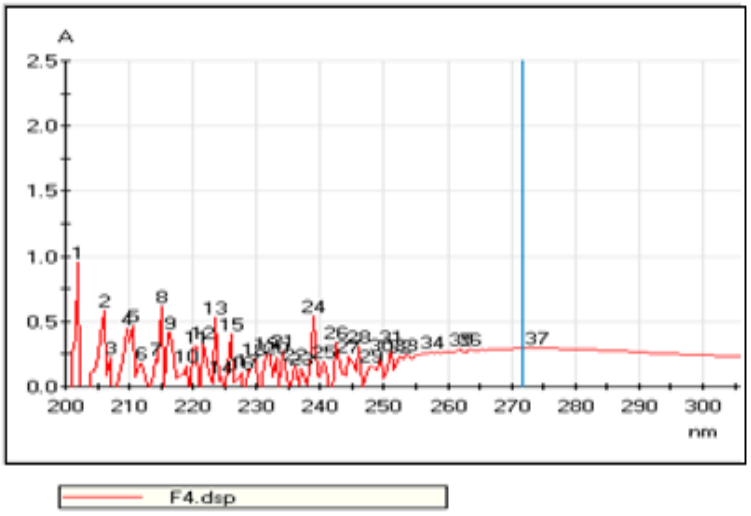

Figure 4: UV spectrum of F4; absorbance vs wavelength of absorption λ (nm)

Scanned between 200-400 nm

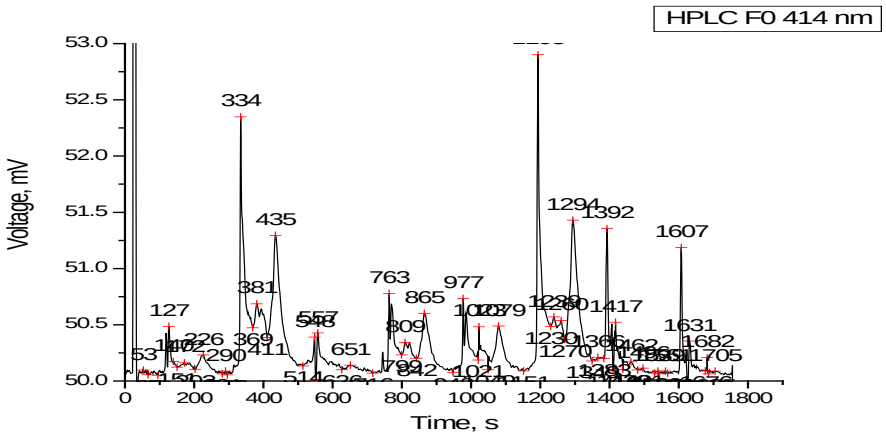

Figure 5: HPLC Chromatogram of the crude extract at detection 414 nm[6].

Table 1-2 emphasis and characterize the different PCD contained in F1 and F4 based on the wavelengths of each peak according to previous results, these findings could represent a database of wavelengths for identification of phytochemicals in plant extracts and will be useful as base of information for further LC-MS, GC and MNR characterizations of F1-F4.

Table 1: Possible phytochemicals contained in F1 characterized by UV spectrophotometry

Peaks N°	Max λ, nm	Possible identified phytocompounds in F1	Similarity references
1	202	Terpenes, Dehydrolinalol , steroidal saponins	[7,8]
2	203	Terpenes, Dehydrolinalol, saponins	[7, 8]
3	205	Terpenes, saponin glycosides	[7, 8]
4	206	Terpenes, Dehydrolinalol , steroidal saponins	[9, 8,7]
5	208	Terpenes, Dehydrolinalol , steroidal saponins	[9, 8,7]
6	210	Terpenes, Dehydrolinalol	[10, 8]
7	212	Cinnamic Ac., Caffeic Ac.	[11, 9]
8	213	Gentisic Ac., *o*- coumaric Ac.	[11,12, 9]
9	214	Mucicdigallate Ac., ferulic Ac.	[11]
10	215	Sterol, Mucicgallate Ac., Isoferulic&caffeic Ac.	[11]
11	217	Chlorogenic Ac. Gallic Ac. , Chebulic Ac.	[11, 9,12,13]
12	220	Sterols, polyterpenes, Gallic Tannin, Caffeic Ac., trigalloylglucose	[12,14, 8]
13	222	Terpenes, Farnesol , caffeoyl -D-glucose	[14,8]
14	224	Terpenes, Farnesol, galloylglucose	[9, 8]
15	225	Terpenes, Farnesol , 3' -hydroxy- 5,7,4' - trimethoxyflavan -3-ol	[8, 9]
16	228	Terpenes, Farnesol	[8]
17	229	Terpenes, Farnesol	[11, 8, 9]
18	230	Terpenes, Farnesol, Alkaloids, saponin	[15, 8]
19	233	Nerol	[11, 8]
20	234	Nerol	[9, 8,16]
21	236	Nerol, Ferulic., isoferulic Ac.	[11, 8, 9]
22	237	Nerol, Ferulic Ac., isoferulic Ac.	[9, 8,15]
23	238	Nerol, Sinapic Ac.	[9, 8]
24	239	Nerol, Steroid 4 -en -3-one	[9, 8]
25	240	Nerol, Steroid 4 -en -3-one	[17, 8, 9]
26	244	Terpenes, Linalool , Sterols	[12, 8]
27	246	Terpenes, Linalool, Chicoric AC., Formononetin , 1,4 benzoquinone	[9, 8,18]
28	248	Terpenes, Linalol, Fisetin	[11, 8, 23]
29	249	Terpenes, Linalool, Formononetin	[24, 8, 9]
30	250	Terpenes, Linalool, Quercetin , Daidzein	[9,8, 25]
31	251	Ganoderic Ac., Ellagic pentose Ac.	[18]
32	252	Ganoderic Ac., Ellagic pentose Ac.	[9,18,13]
33	254	Gallic tannin, Luteolin , QRLCNCKACMC , Alkaloids, Saponin	[19, 20, 14, 21,9, 22]

Ac: Acid

QRLCNCKACMC: Quercetin, Rutin, Luteolin, Chrysin, Naringenin, Catechin, Kaempferol, Apigenin, CaffeicCinnamic Ac., Myricetin, Ac.

Table 2: Possible phytochemicals contained in F4 characterized by UV-Vis spectrophotometry

Peaks N°	Max λ , nm	Possible identified phytocompounds in F4	Similarity references
1	202	Steroidal saponins	[9, 7]
2	205	Glycoside saponines; Bacopasaponin, Jujubogenine	[26]
3	206	Steroidal saponins	[9, 7]
4	209	Steroidal saponins	[9, 7]
5	210	Apigenin, Naringenin , Hesperidin, Quercetinhexoside	[9,10, 9]
6	212	Quercetin pentoside, Caffeic Ac ., Luteolin -6- C- glucoside	[11, 9]
7	214	Quercetin pentoside , Caffeic Ac., Luteolin -6- C- glucoside digallate , ferulic Ac .	[11, 9]
8	215	Steroid, Mucicgallate Ac., Isoferulic&Caffeic Ac ., baicalein	[27, 11]
9	216	3 ', 4 ', 5'- trimethoxyflavone, baicalein -7 -O- glucuronide	[11, 9, 13]
10	219	Vanilic Ac ., galloyl - Hexahydroxydiphenic acid - glucose	[11, 13]
11	220	Flavonoids, sterols &polyterpenes, Gallic Tannin, Caffeic Ac., trigalloylglucose	[14, 28, 8, 11, 20]
12	223	Quercetin hexose	[9]
13	225	3'Hydroxy -5, 7,4' - trimethoxyflavan -3-ol , Mallotusinine	[9, 11]
14	226	Naringenin , *p*-coumaric Ac.	[11, 9]
15	227	Steroid eriodictyol	[11, 27,8, 9]
16	229	Theaflavin , Cyanidin -3 -O- rutinoside , (-) - epicatechin , (-) - epigallocatechin	[9, 11]
17	230	Terpenes, Farnesol , (+) - catechin , Taxifolin , Mesaconitine , Saponin	[9, 8, 15]
18	231	*m* -coumaric Ac., Caffeine, Quercetin , Aconitine , Hypaconitine, Mesaconitine	[9]
19	233	Terpenes, Nerol	[8]
20	234	Terpenes, Nerol , Flavonoids , Quercetin	[9, 8, 29]
21	235	7,3 ' , 4'- Trihydroxyflavone , Catechin, Epicatechin	[17, 9]
22	237	Unidentified	…
23	239	Isoferulic Ac ., Daidzein , Steroids 4 -en -3-one	[9,18]
24	240	Flavonol, 3,5,5-Trimethyl-1,4-cyclohexadion-2-ene	[9, 23]
25	241	Chlorogenic Ac., Steroids 4 -en -3-one	[27, 30]
26	243	Chrysin , Triterpene saponin	[25]
27	245	1,4 naphthoquinone	[18]
28	247	Flavone, Trans- caftaric Ac.	[9, 22]
29	248	Terpenes, linalool , Daidzein -7 -O- glucoside, Fisetin	[9, 8,18]
30	250	Quercetin , Daidzein	[9, 24]
31	252	Flavonoids, Ganoderic Ac., Ellagic pentose Ac.	[13, 31,11]
32	253	Luteolin , Fisetin	[9, 24]
33	254	Gallic Tannin, Luteolin , QRLCNCKACMC , Jesaconitine , Saponin	[14, 20,29, 32, 21,19]
34	258	Quercetin pentoside , isoquercitrin , Furocoumarin : Epoxybergamottine	[9, 31]
35	263	Morine, Anthraquinone	[9, 18]
36	267	Kaempferol , Quercetin hexose	[9, 31]

37	366	Quercetin, flavonoids , Alkaloids ; 2-methoxy-3-hydroxy-10-methyl acridone-1,4- quinone , Chalcone : 1,3-diphenylpropen-3-one	[30, 31]
38	374	Myricetin , 3,3 ', 4 ', 5,7 pentahydroxyflavone	[33, 34]
39	438	Coumarin, Peonidin -3 -O- diglucosidine	[35, 36]
40	486	Cardiac glycosides	[37]
41	513	Delphinidin -3- glucoside, Malvidin -3- glucoside, Delphinidin -3-acetylglucoside, Petunidin -3- acetylglucoside , Malvidin -3 –p coumaroyl glucoside, Petunidin-3-p-coumaroyl glucoside.	[9, 11, 31]
42	520	Delphinidin , Pelargonidin , Cyanidin , Anthocyanin , Orthobenzoquinone	[38, 39,18, 40]
43	630	Catechin	[41]
44	672	Anthocyanin, malvidin -3- glucoside	[42]
45	679	Unidentified	…

202-267 nm (UV, Figure 4), 202-679 nm (UV-Visible, Figure 2)

Table 1-2 attest that F1 and F4 from *Solanum aethiopicum* crude extract (F0) contained several phytocompounds precisely identified base on their wavelengths, the results comply with those indicated[4]**,** however; Table 3 describes precisely the ranges of wavelengths for their detection.

Table 3: Phytocompounds of F1-F4 and ranges of detection wavelength

	Detectable classes of phytocompounds	Physicochemical properties
UV / HPLC detection	**176 – 202 nm** Sterols,Terpenes **202 - 254 nm** Sterols, Terpenes, Phenolic acids, some Saponins and Flavonoids	F1: (mostly lipophylic compounds)
UV-Vis / HPLC detection	**176 – 202 nm** Sterols, Terpenes, Phenolic acids **202 - 254 nm** Sterols, Terpenes, Phenolic acids, Saponins, Flavonoids, few Alkaloids, Coumarins, Quinones **254 – 679 nm** Cardiac glycosides, Tannins, Quinones, classes of Flavonoids, derivatives and glycosides	F4: (lipophylic, hydrophilic, amphiphilic compounds)

It comes out from the recapitulative Table 3 that the different classes of PCD contained in F1-4 are detectable in a certain range of wavelengths and F1 is contained mostly hydrophobic substances while lipophylic, hydrophilic, amphiphilic phytochemicals are embedded in F4.

The effect of F1 and F4 on Jurkat cellshas been performed in order to evaluate preliminary pharmacological activity of these extracts, the experiments were triple duplicated and monitored by microscopy and flow cytometry. Figures 6 and 7 clarify the effect of F1 and F4 on Jurkat cells after 24 and 48 hours treatment using different concentrations of F1 and F4.

After 24 h treatment of the cells with [F1]: 0.3 and 33 µg/ml; [F4]: 15 and 25 mg/ml, Figure 6 shows that the percentage of living cells decreases in the treated cells; 40.2 % of living cells are observed in the cells treated with 0.3 µg/ml of F1 while 36.1 % of the viable are remained after been treated by 33 µg/ml of F1 which represents more than 50 % of dead cells after treatment. The same effect was noticed with cells treated using F4 extract; a decrease in cell viability was noticed; only 0.6 % and 0.3 % of viable cells were remaining upon treatment with 15 and 25 mg/ml of F4.

Flow cytometry and microscopic monitoring of F1 and F4 effects of on Jurkat cells

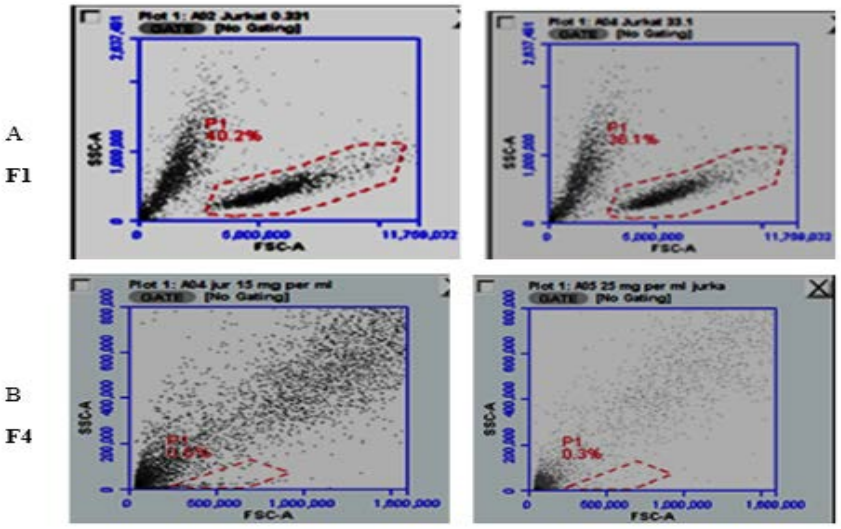

Figure 6: Flow cytometry Dot plots: Anti-proliferative action of F1 and F4 on Jurkat cells

Even though F4 seems to have the most pronounced effect, F1 and F4 induce Jurkat cancer cells deathby affecting their growth and proliferation. More experiments were performed for 48 h treatment and monitored by microscopy in order to practically visualize the action the extracts on Jurkat cells proliferation. Images of Figure 7 show the action of F1 of the cancer cells after 48 h confirming the results of flow cytometry analyses.

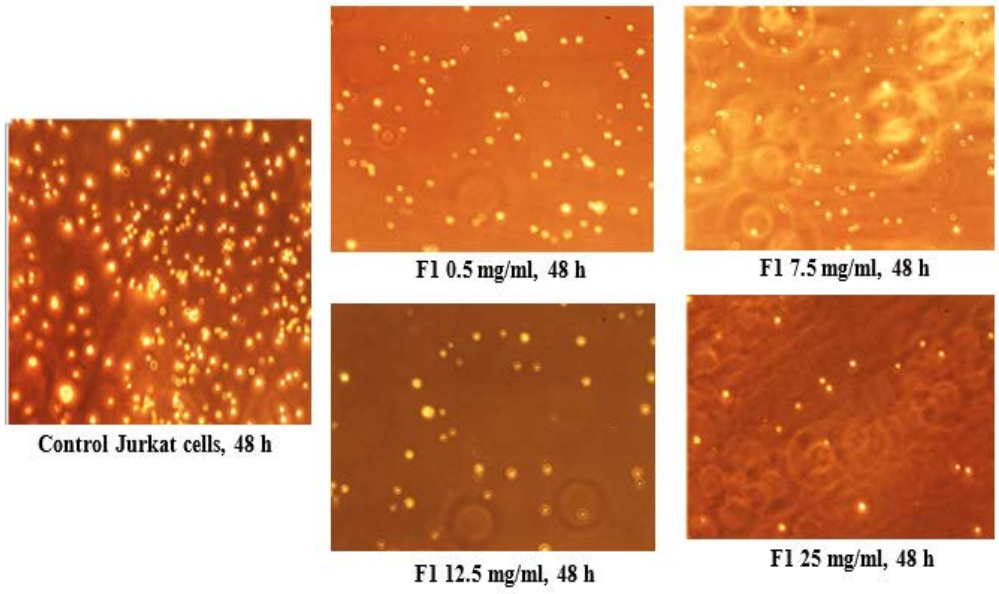

Figure 7: Anti-proliferative effect of F1 on Jurkat cells after 48 h of treatment

Images show that after 48 h of treatment with 0.5, 7.5, 12.5 and 25 mg/ml F1, a decrease in Jurkat cells growth and proliferation was noticedcompared to the control cells (without treatment) where the cells proliferate normally. F1 induces cell death and anti-proliferation activity against the cancer cells concentration-dependently and the highest effect observed incells treated with25 mg/ml, after triple replicate experiment, the same action was also observed on Jurkat cells using 15 and 25 mg/ml of the n-butanol extract[5].

DISCUSSION

In order to accurately identify a single pure compound, HPLC analysis is required whenever possible in addition to UV-Vis spectrophotometry analysis, a different approach apply when it comes to analyzing plant extracts which generally contains several and complex embedded substances. From the findings of UV-Vis spectrophotometry analyses, it is shown that; the majority of phytochemicals of F4 absorb mostly at wavelengths < 500 nm (Figure 1), the richness of F4 compared to F1 is in accordance with the results found by these authors[4] when performing phytochemicals screening on the same extracts.F1 contains mostly steroids and classes of terpenes**;** the results confirm those of TLC[4] and are also similar to those highlighted by[11]who identified steroids and terpenes at the same wavelengths while working of vegetable fruits. From Figure 1, 2 and 4, it is shown that F4 contained at least 36 PCD corresponding to the major peaks revealed 1, 2, 4, 5, 8, 13, 15, 24 which are identified a specific wavelengths. In total; 45 phytochemicals (Table 2) are found in F4 after all analyses and among them 9 absorb only in visible (λ> 400 nm) while 36 are detected in UV. It is advised to consider the nature of the solvent used for each extract when characterizing the embedded PCD, as a matter of fact, hexane extracts are different to n-butanol ones in term of their polarity. Like methanol, n-butanol has the strength to extract all the PCD contained in a plant extract while hexane with a low polarity can just extract a part[6]. Hexane fraction contains mostly hydrophobic PCD such as sterols and terpenes with relatively high $LogP_{ow}$ and hardly water soluble, while n-butanol fraction contains lipophilic, hydrophilic and amphiphilic PCD (Table 3); as example, Quercetin a hardly water soluble and hydrophobic compound has been identified by HPLC at 254 nm against its standard[5] which could be assigned to the compound 33 in Figure 4. It is shown in Table 1 and 2 that most of the PCD of F1 are found in UV while the PCD of F4 are detected both in UV and Visible; F1 is particularly rich in sterol, classes of terpenes and phenolic acids while F4 in addition to these substances contains more polyphenols such as classes of flavonoids, coumarins, quinones, tannins, saponins, some alkaloids and cardiac glycosides as reported by previous findings[8, 9, 11].F1 induces anti-proliferative and apoptotic effects on Jurkat cells as F4 does after 48 h of treatment[5]**.** The observedanti-proliferative effects of F1 and F4 by microscopy confirms those of flow cytometry analyses and both methods seem suitable for cytotoxicity assessment of plant extracts on cancer cells, furthermore, the findings are consistent to the recent results of [43]while working on anticancer activity of *Juglansregia* leaf extract.It is important to notice that according to recent findings, the observed anticancer effects could be related to the richness in terpenes[44], classes of flavonoids[45] of phenolic acids[46], saponins[47], alkaloids[48] and quinones[49] in the tested F1 and F4 as describedby Table 1 and 2.

CONCLUSION:

The current study shows that the phytocompounds contained in the hexane extract absorb mostly at wavelengths between λ176 and 254 while those of F4 are detected from λ176 to 679 nm. sterol, classes of terpene and phenolic acids are the mean PCD found in F1 whereas, in addition to these substances; F4 is especiallyrich in classes of flavonoids, tannins, quinones, saponins, cardiac glycosides and coumarins. The phytocompounds contained in F1 and F4 are probably those responsible for their anticancer against Jurkat cancer cells. The indexed PCD could be further investigated and advised in futuretreatmentof T cells Leukemia cancer, as natural substances are widely used for multiple applications and are expected to be the mean inexhaustible source of new medicines for research and pharmaceutical industries.

Conflict of interest

The author(s) declare that this article has no conflicts of interest.

Acknowledgment

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REFERENCES:

1. Beer.Determination of the absorption of red light in colored liquids. Anna. der Phys. und Chem. 1852; 86:78–88.

2. *Ingle JDJ, Crouch SR. Spectrochemical Analysis. New Jersey: 1st Ed, Prentice Hall. 1988:* 590
3. Grossi M, Di Lecce G; Arru M; Gallina TT, Riccò B. An opto-electronic system for in-situ determination of peroxide value and total phenol content in olive oil. J. Food Eng. 2015; 146: 1–7
4. Mamyrbekova-bekro JA, Boua BB, Kouassi KC, Békro YA. Sur l'analyse qualitative et pharmacologique de 2 plantes antihypertensives utilisées à N'gramanssabo en Côte d'Ivoire. Nat.& Tech.2013; 08 :02-12.
5. Kouassi KC, Mamyrbekova-Bekro JA, Boua BB, Bekro YA.HPLC phytochemical analysis and antiproliferative activity of n-butanolfraction of *Solanum aethiopicum*L. (Solanaceae) from Côte d'Ivoire. Res. J. Rec. Sci. 2015; 4(12): 81-87.
6. Kouassi KC, Mamyrbekova-Bekro JA, Bekro YA. Phytochemical assessment and in vitro diffusion across nasal mucosa of F0 crude extract phytocompounds from *Solanum aethiopicum*L. (Solanaceae). J. Adv. Pharma. Res. 2016; 7(2): 41 – 48.
7. Mi-Jeong A, Jinwoong K. Identification and Quantification of Steroidal Saponins in *Polygonatum Species* by HPLC/ESI/MS. Arch. Pharm. Res. 2005; 28: 592-597.
8. Borzelleca JF, Boobis AR. Toxicologic and Dermatologic Assessment of Cyclic and Non-Cyclic Terpene Alcohols. Food and Chem. Tox. 2008; 46: 237
9. Hung-JC, Baskaran SI, Bing-HC. Determination of Phenolic Acids and Flavonoids in *Taraxacumformosanum* by Liquid Chromatography-Tandem Mass Spectrometry Coupled with a Post-Column Derivatization Technique. Int. J. Mol. Sci. 2012; 13: 260-285
10. Pedro DS**,** Vanessa E**,** Maïté C-P**,** Catherine S**,** Isabelle R**,** Corinne R**,**Frédéric G.High toxicity and specificity of the saponin 3-GlcA-28-AraRhaxyl-medicagenate, from *Medicagotruncatula* seeds, for *Sitophilus oryzae*.*BMC Chem. Biol*. 2012; 12: 3.
11. Hiroyuki S, Yoshinori H, Satoshi N, Hitoshi A, Kazuki K. Simultaneous Determination of All Polyphenols in Vegetables Fruits, and Teas. J Agric Food Chem. 2003; 51(3): 571-81
12. Rebecca Robbins J. Phenolic Acids in Foods: An Overview of Analytical Methodology.J. Agric. Food Chem.2003; 51 (10): 2866-2887
13. Gülçin SÇ, Özlem BA. Column Chromatography for Terpenoids and Flavonoids, Chromatography and Its Applications. InTech. 2012. ISBN: 978-953-51-0357-8.
14. Abd El-Mawla AM. Cardiac glycosides from shoot cultures of *Cryptostegiagrandiflora*.Phar. Res. 2010; 2:15-8.
15. Jagmohan SN, Pramod S, Rawat SM. High-performance liquid chromatography analysis of plant saponins: An update 2005-2010. Pharm. rev. 2011; 5(10):155-158.
16. Dejan G, Vele T, Milovan V, Ljubodrag V, Vlatka V, Slobodan M. Polyphenolic compounds in seeds from some grape cultivars grown in Serbia. J Serb. Chem. Soc. 2010; 75 (12): 1641–1652
17. Won JC, Moon HP, Gyoung WK, Joung HK, You JS. Determination of Catechin Compounds in Korean Green Tea Infusions under Various Extraction Conditions by High Performance Liquid Chromatography. Bull. Kor. Chem. Soc. 2005; 26: 747-754.
18. Rose MC, Diane M, Knight MC. Fluorescence Spectroscopy Reveals Ubiquitous Presence of Oxidized and Reduced Quinones in Dissolved Organic Matter. Environ. Sci. Technol. 2005; 39: 8142-8149.
19. Veena S, RituP.Isolation and characterization of saponins from *Moringaoleifera* (moringaeceae) pods. Int J Pharm Pharm Sci. 2013; 5: 179-183.
20. Ann E. Hagerman. HPLC of Gallotanninsfrom original method (Hagerman and Butler, J. Biol. Chem. 256, 4494-4497, 1981). 2002; 1.
21. Krystyna P, Magdalena B. Analysis of phenolic acids and flavonoids in honey.Tren. Anal. Chem. 2009; 28(7): 893-902
22. Rebula CV, Branka M, Irma T, Andreja Š, Polonca T. Determination of Polyphenols in White Grape Berries. ActaChim. Slov. 2006; 53: 58–64
23. Zarghami NS, Heinz DE. Monoterpene aldehydes and isophorone- related compounds of saffron. Phytochem. 1971; 10: 2755- 2761
24. Yanguo S, Xi Z, Xingya X, Yan Z, Hongbin X, Xinmiao L. Rapid Identification of Polyphenol C-Glycosides from *Swertiafranchetiana* by HPLC–ESI-MS–MS. J Chr. Sci. 2009; 47:190-196.
25. Qiang F, Ke Z, Ming-Bo Z, Si-Xiang Z, She-Po S, Yong J, Peng-Fei T. Three new triterpene

saponins from *Clematis chinensis*. J. Asi. Nat. Prod. Res. 2013; 15(6): 610–618

26. Watoo P, Sakchai W, Kanchalee J, Waraporn P, Hiroyuki T, Kornkanok. Determination of Saponin Glycosides in *Bacopamonnieri* by Reversed HPLC. Thai. Pharm. Health Sci. J. 2007; 2(1):26-32

27. Alexander K, Milos B, William Griffiths J. Spectroscopic Methods of Steroid Analysis. Springer. 2nd ed. 2010; 22: 1224

28. Eugene NO, Jude CI, Catherine CI, Augustine AU.Quantitative High Performance Liquid Chromatographic Analysis of Simple Terpenes, Carotenoids, Phytosterols and Flavonoids in the Leaves of *Acalyphawilkesiana*Muell Arg. Pac. J. Sci.Tech. 2010; 11: 480-487

29. Dimitrios T, Martina S, George P, Vassiliki O. Characterization of Flavonoid Subgroups and Hydroxy Substitution by HPLC-MS/MS.Molecules. 2007; 12: 593-606.

30. Mirza B., Vicente SH, Darija Š, Željan M. Determination of Flavonoids, Phenolic Acids, and Xanthines in Mate Tea (*Ilex paraguariensis* St.-Hil.). J. Anal. Meth. Chem. 2013; 6.

31. Davide B, Ersilia B, Corrado C, Ugo L, Giuseppe G. Distribution of C- and O-glycosyl flavonoids, (3-hydroxy-3-methylglutaryl) glycosyl flavanones and furocoumarins in *Citrus aurantium* L. juice. F. Chem. 2011; 124: 576–582

32. Yuichi N, Kaori Y, Toshihiro K, Makoto I, Yuichi K, Kazuaki N, Susumu T, Shuichi T, Hiroshi S, Keiko A, Minoru O, Setsuko S, Motoyoshi S, Yukihiro G. Physicochemical quality evaluation of natural compounds isolated from crude drugs. J Nat Med. 2006; 60:285–294.

33. YaoLH, JiangYM, Shi J, Tomas-BarberFA, DattaNR, Singanusong, Chen SS. Flavonoids in Food and Their Health Benefits. P. F. Hum. Nut. 2004; 59: 113–122

34. Urmanova FF, KomilovKh M. F l a v o n o i d s of *Achilleasantolina*. Chem. Nat. Comp. 1999; 35:214

35. Fei H, Lin M, Guo-Liang Y, Na-Na L, Qiu-Hong P, Jun W, Malcolm JR, Chang-Qing D. Biosynthesis of Anthocyanins and Their Regulation in Colored Grapes. Molecules. 2010; 15: 9057-9091.

36. www.exciton.com. Coumarin 440. Tel: 937.252.2989, Fax: 937.258.3937. Dayton, OH 45437. E-mail: info@exciton.com , 18-07-2013

37. Sohch P, Sedhakovs V, KarliiFekR. Spectrophotometric determination of cardiac glycosides by flow-injection analysis. Anal. Chem Act. 1992; 269:199-203.

38. James MH, Robert FD, Gary RB, Joanne M., Holden, David BH, Haytowitz Seema, Bhagwat, Susan G. Flavonoid Content of U.S. Fruits, Vegetables, and Nuts. J Agric. Food Chem. 2006; 54: 9966−9977.

39. Josep V, Silvia M, Pilar M, Eva B, Luís A. Advanced separation methods of food anthocyanins, isoflavones and flavanols.J. Chroma. 2009; 7143–7172.

40. Alfred MEIER R, Georges H. The long-wavelength MCD of some quinones and its interpretation by semi-empirical MO methods. Chem. Phy. 1987; 113: 287-307.

41. Miyazawa T, Nakagawa K.Structure-related Emission Spectrometric Analysis of the Chemiluminescence of Catechins, Theaflavins and Anthocyanins. Biosci. Biotech. Biochem. 1998; 62: 829-832

42. Maria Josephine B.The color of red wine. PhD Thesis,Adelaide University, Australia. 2007; 306

43. Wei L , De-Yuan L, Hai-Dong W, Zhe-Jun Z, Jie H, Zong-Zhe L. Juglansregia Hexane Extract Exerts Antitumor Effect, Apoptosis Induction and Cell Circle Arrest in Prostate Cancer Cells In vitro. Trop. J. Pharma. Res. 2015; 14 (3): 399-405

44. Wang GH, Ahmed AF, Sheu JH, Duh CY, Shen YC, Wang LT. Suberosols A–D, four new sesquiterpenes with β-caryophyllene skeletons from a Taiwanese gorgonian coral *Subergorgiasuberosa.* J Nat Prod. 2002; 65:887–91.

45. Maheeps K, Sharma CN, Dobhal MP, Joshi YC. Flavonoids: A versatile source of anticancer drugs. Pharma. Rev. 2011; 5(9): 1–12.

46. Thanaset S**,** Suwatchai M**,** Somprasong K**,** Suporn N**,** Prasan S**,** Chanokbhorn P**,** Arpa S, Banchob S**.** Histone deacetylase (HDAC) inhibitory and antiproliferative activities of phenolic-rich extracts derived from the rhizome of Hydnophytumformicarum Jack. sinapinic acid acts as HDAC inhibitor. BMC CAMed. 2013; 13:232.

47. Khanna VG, Kannabiran K. Anticancer-cytotoxic activity of saponins isolated from the leaves of *Gymnemasylvestre* and *Ecliptaprostrataon* HeLa cells. Int J Green Pharm. 2009; 3:227-9.

48. Habermehl D**,** Kammerer B**,**Handrick R**,** Eldh T**,** Gruber C, CordesN**,** Daniel P T**,** PlasswilmL**,** BambergM**,** Belka C, Jendrossek V.Proapoptotic activity of Ukrain is based on Chelidoniummajus L. alkaloids and mediated via a mitochondrial death pathway. *BMC Can.* 2006; 6:14-22

49. Tambama P, Abegaz B, Mukanganyama S. Antiproliferative Activity of the Isofuranonaphthoquinone Isolated from *Bulbinefrutescens* against Jurkat T Cells. BioMed Res. Int. 2014; 14.