Research Journal of Recent Sciences _____ ISSN 2277-2502
Vol. **4(12),** 81-87, December **(2015)** *Res.J.Recent Sci.*

HPLC phytochemical analysis and antiproliferative activity of *n*-butanol fraction of *Solanum aethiopicum* L. (Solanaceae) from Côte d'Ivoire

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Available online at: www.isca.in, www.isca.me
Received 24th July 2014, revised 5th December 2014, accepted 8th May 2015

Abstract

Solanum aethiopicum L. (Solanaceae) commonly called Aubergine, is a popular plant and one of the most eaten as a leaf and vegetable. Due to the lack of phytochemical and biological information on the species of Côte d'Ivoire, the goal of our previous investigations was mainly a qualitative analysis by TLC and anti-hypertensive activity of organic extracts from the flower stalk. This research has highlighted the richness of the plant in different secondary metabolites and an efficient in vivo hypotensive activity. The aim of the present work was to certify by HPLC analyzes, the phytochemical composition of n- butanol fraction (F4) of S. aethiopicum flower stalk obtained from the methanol extract and study its anticancer activity on Jurkat cell lines. The results have confirmed our previous findings in relation to the diversity of bioactive phytocompounds contained in S. aethiopicum flower stalk; on one hand, Quercetin identified in F4 at a retention time of 2.65 min and on the other hand, F4 induces in vitro antiproliferative and apoptotic activities on Jurkat cells with IC_{50}*= 11.77 ± 2.4 mg/ml.*

Keywords: Phytochemical analysis, HPLC, antiproliferative activity, *Solanum aethiopicum*, Côte d'Ivoire.

Introduction

Well known in African traditions, plants are not only sources of nutrition but also represent huge therapeutic reserves of phytochemicals; secondary metabolites which are always been described as undeniable sources of nutrients and drugs[1].

The granted importance to secondary metabolites is certainly the fact that they are endowed with variable therapeutic properties. They may, for example, protect against the arising risks of several diseases such as chronic, cardiovascular and neurodegenerative diseases and cancers[2,3]. Polyphenols are used in pharmaceutical industries and represent a great hope; however, much remains to be done for a deeper understanding of their physicochemical properties in order to increase their bioavailability. *Solanum aethiopicum* L. (SA) is a popular plant whose fruit and leaves are very consumed in Africa and Asia[4] and in addition to its nutritional value, its organs are interesting sources of bioactive chemicals effective for the treatment of several human diseases[6,7] but chemical investigations and therapeutic properties of the flower stalk widely used by Ivorian traditherapists are still unknown.

In 2012, chemical and pharmacological studies on the flower stalk of *S. aethiopicum* revealed its richness in various classes of secondary metabolites with significant antioxidant activities and reported in *vivo* dose - dependent anti-hypertensive properties on rabbit blood pressure[7]. The dual aim of this work was firstly; to reveal all the phytochemical compounds contained in *n*-butanol organic fraction (F4) obtained from the flower stalk of S. *aethiopicum* by HPLC analyzes and secondly; to assess their anticancer potential on Jurkat leukemia cell lines.

Material and Methods

Preparation of the plant material: The plant material was composed of floral peduncle (flower stalk) of S. *aethiopicum*. The organ was harvested in Assoumoukro (Côte d'Ivoire) thanks to ethno pharmacological information collected from Mr. Kabran KB, Traditherapist; then identified in accordance with the specimen of the herbarium at National Centre of Floristic (CNF) of Felix Houphouët-Boigny University (Cocody-Abidjan) by Professor AKE-ASSI Laurent[7]. The flower stalk has been cleaned, dried under constant airing for a week at Laboratory of Bioorganic Chemistry and Natural Products of Nangui Abrogoua University then finely pulverized using an electric grinder (Retsch Brand, Type SM 100).

Preparation of crude extracts: 200 g of plant (flower stalk) powder was macerated in a mixture methanol-water (8:2, v / v) for 24 h. After vacuum filtration, the macerated solution was concentrated using a rotavap under reduced pressure and the methanol extract suspended in water. After a successive

fractionation by hexane, chloroform, ethyl acetate and *n*-butanol, the obtained fractions; (F1 for hexane, F2 for chloroform, F3 (ethyl acetate) and F4 (*n*-butanol)) were dried after distillation of the solvents and the extracts well conserved for the different tests[8,9]. For the following experiments, only *n*-butanol (F4) fraction obtained from the methanol extract was used for phytochemical characterization by HPLC and cytotoxicity tests, as *n*-butanol fractions have been widely reported to have both interesting antioxidant properties and significant anticancer activities on various types of human cancers[10-13].

Phytochemical characterization of F4: Phytochemical analysis of *S. aethiopicum* flower stalk (*n*-butanol fraction) was performed at 254 nm by modified HPLC method[14] and by UV-Visible spectrophotometry. HPLC type Varian 9012 Solvent Delivery System (Thermo Separation Products, USA) equipped with a Column C_{18} Gemini 5µ 110 R, Phenomenex and Part N°: 00E-0152-E0, 100 x 4.6 mm. UV-Vis detector D107 from Thermo separation products, automatic sample injector type Dynamax AI-1A of Rainin were used. As conditions; the mobile phase used: MeOH / H_2O; 65:35 (v/v), 1% Acetic Acid, (B): ACN 65% in water, flow rate (2 ml/min) and sample volume injected of 20 µl. The detection (254 nm), T = 25 °C and the Program Picolog Technology were used. UV-visible scan spectrophotometer type Varian Cary 50 BIO Australia, Pty Ltd use and Quercetin (95% purity, CAS N°: 117-39-5 from Sigma Aldrich, Sweden). Methanol (Lot: SZBC272MV, CAS N°: 67-56-1, Chromasolv for HPLC ≥ 99.9% purchased from Sigma Aldrich, UK) and Acetic Acid (code: 2000211329, Schardau, Spain).

Cell culture: Jurkat cells are human cell lines of acute lymphoblastic leukemia of T cells derived from peripheral cells of a 15 years old patient with acute T-cell leukemia[15] in 1974. Jurkat cell line from ATCC-LGC (LGC Standards, Teddington, Middlesex, UK) was obtained from the Department of Biomedical science, Malmö University, Sweden. The cells were maintained in a formulated RPMI-1640 medium ATCC (American Type Culture Collection) according to Invitrogen (Catalog 2010–2011, Life Technologies) and Promega protocols, supplemented with 10% BSA (Bovine Serum Albumin, Gibco) then cultured at 37°C with 5% CO_2 in a humidified atmosphere.

MTS cytotoxicity test: MTS test is a calometric and non-destructive technic frequently used to evaluate cells viability, proliferation and cytoxicity of drugs on cells. By reduction of MTS in Formazan, the absorbance read at 490 nm is proportional to cell viability and a consequent decrease of treated cells compared to controls indicates an anti-proliferative effect of the drug used. $0.5x10^6$ viable Jurkat cells were used in 96 wells plates round bottom (Sarstedt inc. (Newton, USA)). After the treatment of the cell suspensions (RPMI-1640) using 10, 15, 25 and 50 mg/ml of F4 (RPMI-1640), all the cells including the controls (untreated) were incubated under 37°C during 24 and 48 hours. MTS assay was performed by adding 20 µl of MTS/PMS solution (The CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay, Promega Corporation, Madison, USA) to all the wells, after 2 h of incubation, the absorbance was measured at 490 nm using BIO-TEK® micro plate reader. The experiments were performed 3 times.

Statistical Analysis: Statistical analysis was made using Dunnett test by performing Student's t-statistic with each treatment compared to the controls for 2 days experiments. Using as theoretical value $\alpha = 0.05$. Differences are considered significant between compared mean values when the probability (P) associated with the statistic is $P < \alpha$ and not significant when $P \geq \alpha$.

Results and Discussion

The phytochemical characterization of *n*-butanol fraction of S. *aethiopicum* floral stalk (F4) by HPLC was performed at 254 nm. F4 was dissolved in MeOH 99.9% (1:1) and filtered 3 times using Acro 50 Filter (0.45µm) and the HPLC calibrated before all analyzes. The results (figure-1) show that F4 contains different types of phytocompounds with specific retention times; this diversity in bioactive substances is in accordance to our previous reported TLC investigations[7] in relation to the richness of F3 and F4 in phenolic compounds including flavonoids. After performing HPLC analyzes at 254 nm of different standards solutions in the same experimental conditions, it is more likely that the compound of F4 with a retention time of 159 s (2.65 min) could be Quercetin identified at the same retention time (figure-2).

Mobile phase: MeOH / H_2O; 65:35 (v/v), 1% Acetic Acid, flow rate: 2 ml/min, sample volume injected: 20 µl, 25 °C, detector wavelength set at 254 nm. The retention time of Quercetin found at 2.65 min in this qualitative study is similar to those of Shanmugam et *al*., who obtained 2.6 min as retention time in their investigations and, that in the same order with the results of Jelena *et al*., who found a retention time of 2.8 min for Quercetin while working on Quercetin and Rutin standard solutions[16,17]. It is noticed that in addition to Quercetin, F4 contains other phytochemical compounds (figure-1) of interest and further investigations are going on for their identification and thereafter, investigate their potential pharmacological activities. Afterwards, Quercetin standard solution has been analyzed by UV-Visible spectrophotometry; scanned between 200 and 800 nm and the result is showed by the following figure-3.

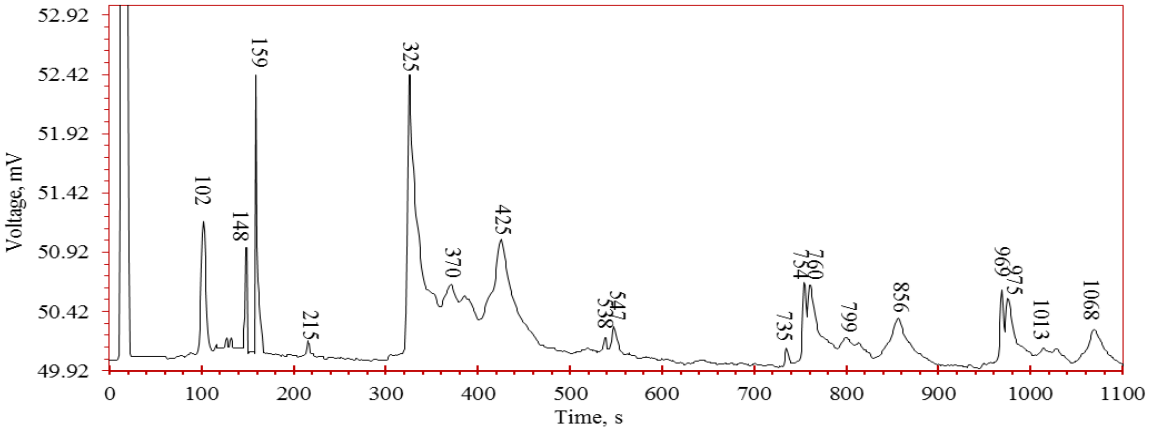

Figure-1
HPLC Chromatogram of *S. aethiopicum* flower stalk *n*-butanol fraction (F4) at 254 nm

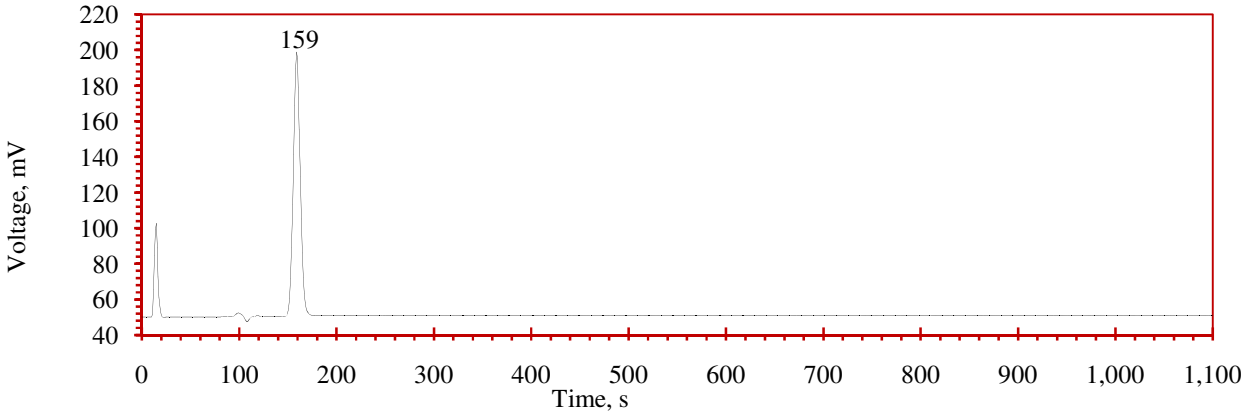

Figure-2
Chromatogram of Quercetin standard solution at 254 nm, (40 µg/ml, area= 1177 ± 0.002)

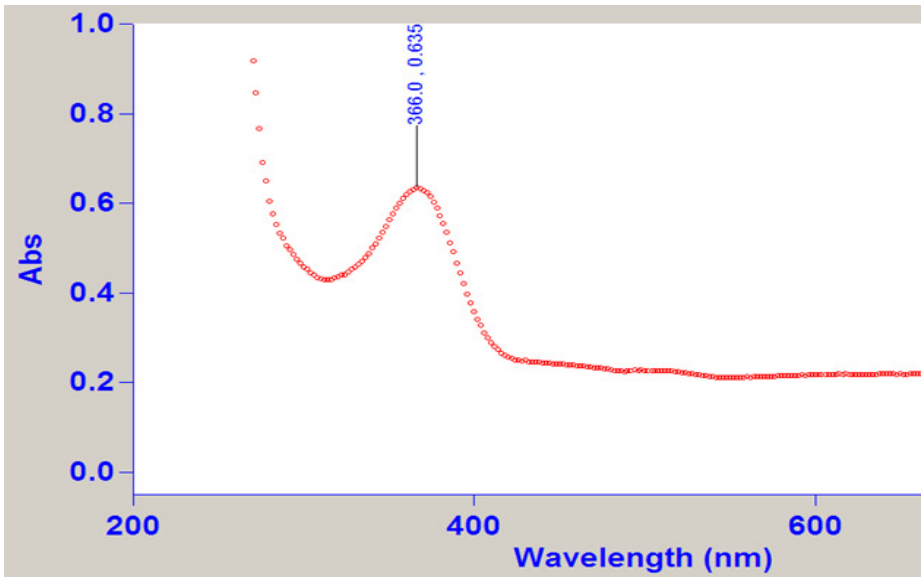

Figure-3
Quercetin standard spectrum by scanning UV-Visible spectrophotometry

It is found that the spectrum of Quercetin has 2 maxima at 254 nm (Absorbance > 1) and at 366 nm. Similar results had been highlighted by Timbola *et al*., while working on Quercetin solutions[18]. Moreover, in 2010, Kumaria *et al*.[19] found by UV-Vis analyzes 2 maxima for Quercetin spectrum at 257 and 373 nm which are very close to 254 and 366 nm observed in the present study. In order to evaluate the cytotoxicity activity of *n*-butanol (F4), 0.5 x 10^6 Jurkat cells/ ml were used for the MTS test. 3 replicate experiments of each concentration (10, 15, 25, 50 mg/ml) including controls were performed in 96 well plates for 24 and 48 hours. The absorbance, expressing cell viability read at 490 nm using an ELISA plate reader from BioTek, program Junior KC. The activity of F4 on Jurkat cell lines is described by the following table and figures.

MTS tests show an inhibition of Jurkat cells proliferation after 24 and 48 hours of treatments with different concentrations of F4, these concentration dependent activities are described by figure-4 when considering controls cells as 100 % of cell viability.

After 24 h, using 10, 15, 25, 50 mg/ ml of F4 on Jurkat cells, it was noticed a decrease in cell viability of about 63.3 to 39.6% compared to the controls (untreated) cells. Furthermore, the effect of F4 was more pronounced after 48 h of treatment where a significant antiproliferative activity was observed on treated cells with 19 to 6.37% of viable cells and the concentration able to induce 50 % of cell inhibition or death (IC_{50}) estimated (figure-5).

All the experiments were performed 3 times and values are presented as Mean ± Standard Deviation. Compared with control cells, F4 induces a significant anti-proliferative and apoptotic effects on Jurkat cells after 2 days of treatment with an IC_{50} of 11.77 ± 2.4 mg/ml. The effects of F4 on Jurkat cells have been closely observed and followed (figure-6) using an optical Microscope (x 400) model CKX41SF, Olympus Optical Co. LTD, Tokyo, Japon.

Table-1
Cytotoxic activity of S. *aethiopicum* flower stalk F4 on Jurkat cell lines

[F4], mg/ml	Log [F4]	Abs[a] ± SD, 24 h	Abs[a] ± SD, 48 h
10	1.0	0.679 ± 0.113	0.340 ± 0.028
15	1.2	0.580 ± 0.117	0.201 ± 0.195
25	1.4	0.504 ± 0.168	0.125 ± 0.068
50	1.7	0.425 ± 0.157	0.114 ± 0.069
Control cells[b]	-	1.072 ± 0.058	1.789 ± 0.028

[a] mean absorbance of treated cells ± SD, [b] mean absorbance of untreated cells ± SD, n = 3. At 490 nm, absorbance expresses cell viability.

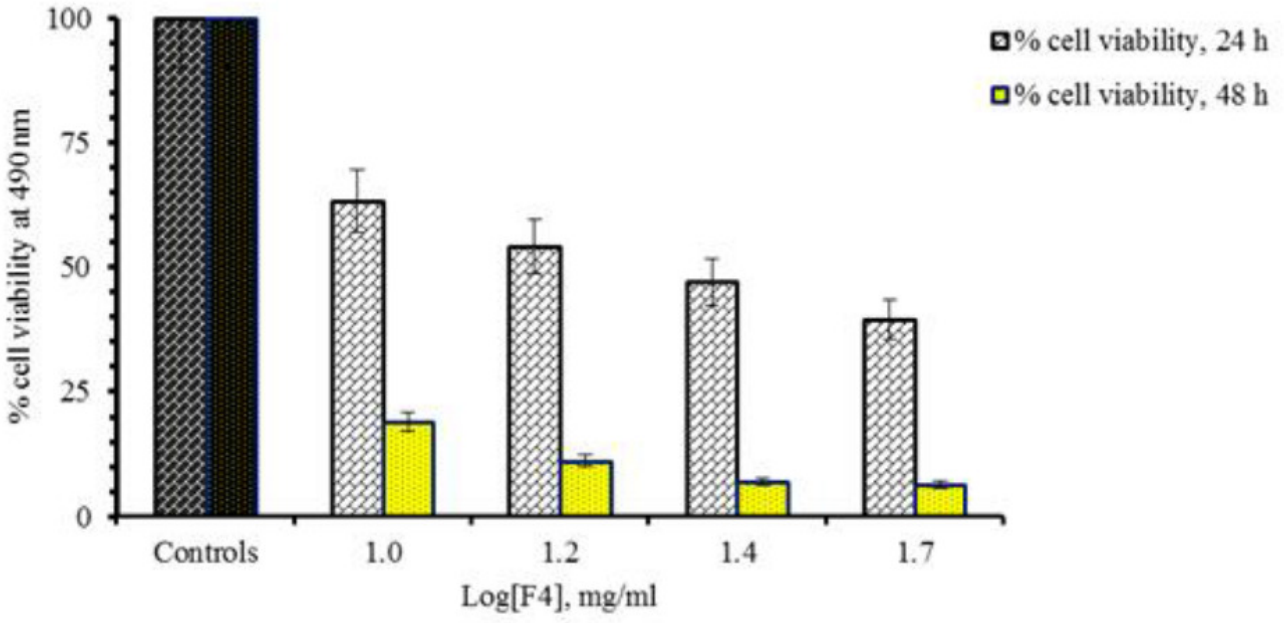

Figure-4
Effect of S. *aethiopicum n*-butanol fraction F4 on Jurkat cells viability

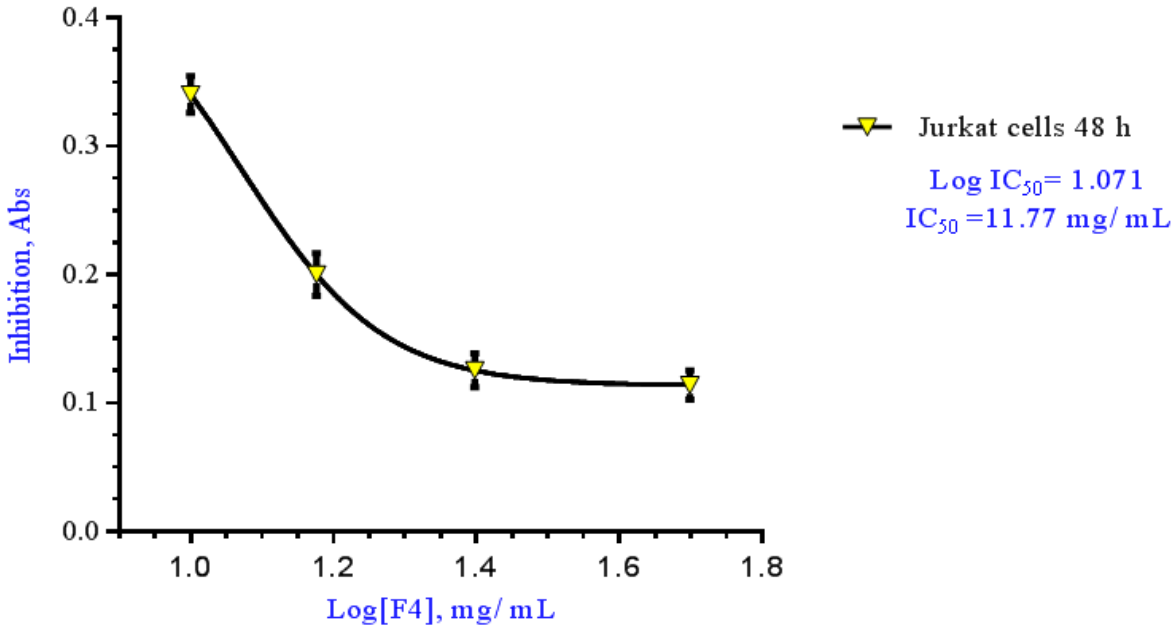

Figure-5
Cytotoxicity activity of S. *aethiopicum* flower stalk F4 on Jurkat cells after 48 h

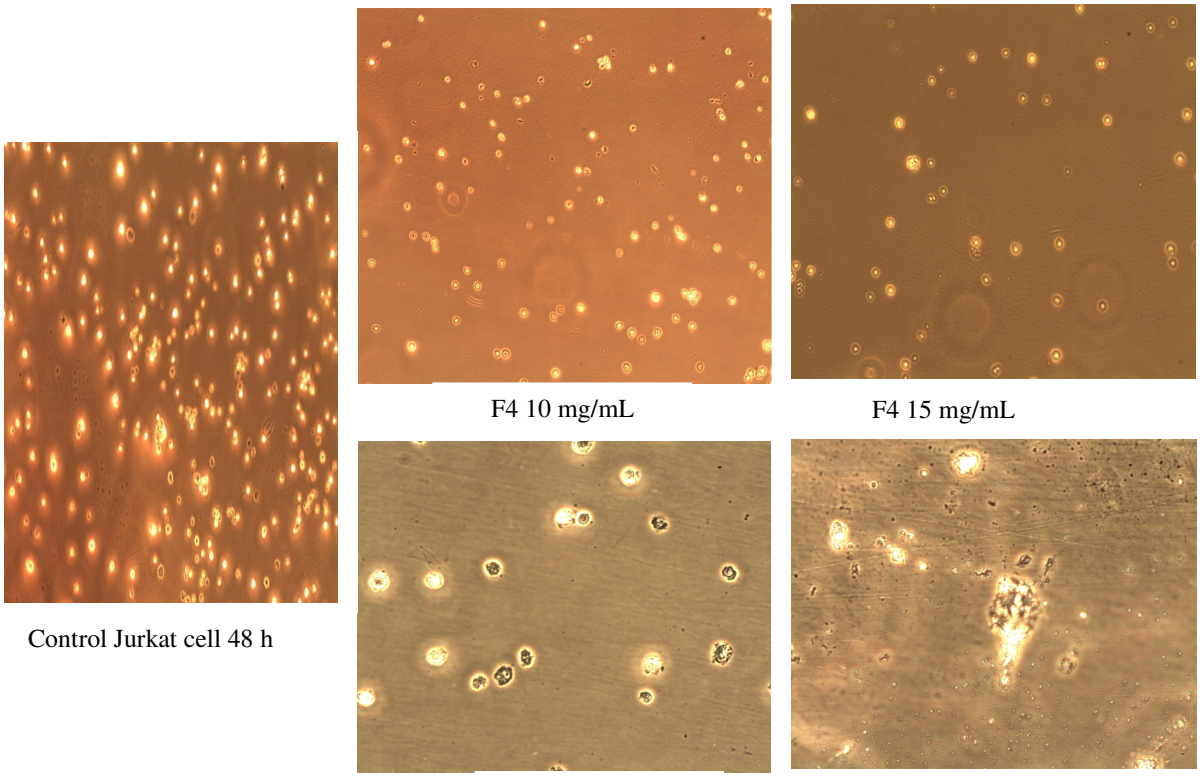

Figure-6
Anti-proliferative and apoptotic effects of F4 on Jurkat cells after 48 h of treatment

Results show that F4 has interesting anticancer activities by reducing significantly cells growth, proliferation and inducing their death compared to untreated cells. The effectiveness of F4 on cancer cells and various human diseases, its richness in phytochemicals including flavonoids like Quercetin had also been reported by recent investigations; they identified Quercetin in *n*-butanol fractions and concluded with the anti-diabetic and anticancer potential of these fractions[20, 21].

Conclusion

The present investigation showed that *n*-butanol (F4) fraction of *Solanum aethiopicum* L. flower stalk, harvested in Côte d'Ivoire is a valuable source of phytochemicals including Quercetin likely identified at a retention time of 2.65 min. F4 induces anti-proliferative and apoptotic activities on Jurkat cancer cells with an IC_{50}= 11.77 ± 2.4 mg/ml. These properties could be related to the richness of F4 in promising bioactive substances which could be proposed as alternative treatment for acute T cell leukemia. In addition to Quercetin, further investigations are ongoing in order to identify all the phytochemicals contained in F4 and investigate their potential pharmacological activities.

Acknowledgement

We are very grateful to Mundus project for the contribution, thankful to Malmö University for the technical support and honor Professor AKE-ASSI Laurent and Mr. Kabran KB for *S. aethiopicum* identification.

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