Phytochemical composition, antioxidant and antiproliferative activities of

*Rosmarinus officinalis* leaves

Winfred Nassazi\(^a\), Isaac O. K'Owino\(^b\), Jacqueline Makatiani\(^c\), Sabina Wachira\(^d\)

\(^a\) Department of Chemistry and Biochemistry, Moi University, Eldoret, Kenya.

\(^b\) Department of Pure and Applied Chemistry, Masinde Muliro University of Science and Technology, Kakamega, Kenya.

\(^c\) Department of Biological Sciences, Moi University, Eldoret, Kenya.

\(^d\) Kenya Medical Research Institute (KEMRI), Centre for Traditional Medicine and Drug Research, Nairobi, Kenya.

\textit{winnipeace15@gmail.com, wnassazi15@gmail.com}

**Keywords:** Polyphenols, cancer, cytotoxicity, prostate adenocarcinoma.

Phytochemicals in *Rosmarinus officinalis* leaves, their total phenolic content, antioxidant potential and antiproliferative activity against human prostate (DU145), colon (CT26) and cervical (HeLa 229) cancer cells were investigated. Extraction was done separately using hexane, dichloromethane, ethyl acetate and methanol. A total of 32 compounds were identified, eight of which were reported for the first time. The highest phenolic content was 476.80 ± 0.69 µg/ml for the methanolic extract which also had the highest antioxidant activity with a minimum inhibitory concentration of 5.39 ± 0.09 mg/ml. Extracts exhibited the highest toxicity against prostate cancer cells and the least against cervical cancer cells.

**Introduction**

Cancer is one of the leading causes of mortality worldwide [1]. It is characterized by irregular proliferation of malignant cells in a series of stages with different biochemical, molecular and cellular events [2]. Cancer is caused by both internal factors (such as mutations, hormones and immune conditions) and external factors like chemicals, radiation and infectious microorganisms [3, 4].

Treatment of cancer is costly, and this has been exacerbated by the resistance of tumor cells to the available antineoplastic drugs. Due to their lack of specificity, the conventional cancer therapies present severe side effects and in most developing countries are inaccessible to cancer patients [5]. Thus, traditional medicine is gaining more attention in chemoprotective management of cancer [1]. Over 3,000 plant species have been reported to have anticancer properties [6]. An example is *Camptotheca acuminate* from which the anticancer drug Camptothecin has been developed [6].

150
Although a number of plants have been claimed to have antitumor properties, they have not been fully investigated for the development of novel anticancer drugs [7]. *Rosmarinus officinalis* L. (Rosemary) is one of the plants used in the traditional management of cancer in Uganda. However, its safety to humans as well as identification and isolation of the main phenolic compounds as the presupposed source of anticancer activity has not been fully documented. In the current study, we report on the phytochemicals in the leaves of *R. officinalis*, their total phenolic content, antioxidant potential and antiproliferative activity against human prostate (DU145), colon (CT26) and cervical (HeLa 229) cancer cells.

**Experimental part**

*Ethical approval*

This study was approved by Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute Scientific and Ethics Review Unit, Kenya (Approval No. KEMRI/RES/7/3/1).

*Sampling and sample preparation*

Leaves of *R. officinalis* were collected from cultivated plants in Wakiso district of Uganda (0°23’36” N, 33°0’9” E) with permission from Uganda Natural Chemotherapeutics Research Institute, Kampala, Uganda where they were identified by Kyoshabire Medius (a taxonomist). A voucher sample (No. 50907) was deposited at Makerere University Herbarium, Kampala, Uganda on 7th August 2019.

Laboratory samples were air-dried in mesh bags and ground into a fine powder using a laboratory mill. Weighed 150 ± 0.1 g of the powder were separately extracted with 775 ml of *n*-hexane, dichloromethane (DCM), ethyl acetate and methanol in 1000 ml conical flasks for 96 hours at room temperature. The crude extracts were filtered using a cheese cloth, Whatman No. 1 filter paper and concentrated to dryness on a rotary evaporator (Rotavapor BUCHIR-100, Switzerland) [8]. The extracts were transferred into sample bottles which were placed in a desiccator of anhydrous sodium sulphate. The yields of the extracts were calculated (*Equation 1*) and they were transferred into tightly stoppered bottles which were kept at 4 °C.

\[
\text{Percentage yield} = \left( \frac{A}{A_0} \right) \times 100
\]

Where \(A\) is the amount of crude extract obtained after drying and \(A_0\) is the weight of the leaves used for extraction.

Fractionation was done for the methanol extract because it had the highest yield. The dried crude methanol extract was divided into two parts; one portion (1.5 g) was kept in the crude form and the other portion (8 g) was subjected to column chromatography fractionation.

*Antiproliferative activity of the extracts*

The Vero, prostate (DU145), colorectal (CT26) and cervical (HeLa 229) cancer cells were
separately thawed in a water bath at 37 °C. Growth media (20 ml) was added to 1 ml of each of the cell lines in T-75 culture flasks and incubated at 5% carbon dioxide and 37 °C to revive the cells. Culturing was done for three days until when the cells obtained at least 80% confluence. The excess media was poured off, leaving the cells attached to the surface of the flask and the flask was washed 3 times with phosphate buffer saline (PBS). Excess PBS was poured off and then 500 µL of Trypsin-EDTA was added into the flask having cells attached to the surface. This was spread evenly on the inner surface of the flask by tilting the flask back and forth and then incubating for 3 minutes. Trypsin was added to detach the cells off the surface of the flask. Growth media (10 ml) was added immediately to stop the action of Trypsin. Growth media was purged gently to allow breaking of clumps between cells.

*In vitro* antiproliferative assay was done for both crude extracts and the solid phase extracted methanolic isolates using MTT assay [9]. Briefly, the cancer cells were washed 3 times with 5 ml of PBS after attainment of 100% confluence and harvested by trypsinization. The number of viable cells was determined by Trypan blue exclusion test. Approximately 2 × 10^4 cells/ml suspension of both Vero and cancer cells were seeded in 96-well plates and incubated for 24 hours. Measured 15 µl of the extracts and the commercial drug doxorubicin at seven different concentrations (1000, 333.33, 111.11, 37.03, 12.34, 4.11 and 1.37 µg/ml) were added from rows H to B and the plates incubated for 48 hours. Row A acted as the negative control (extracts or the drug were not added to it).

After incubation, 10 µl of MTT dye solution was added to each of the wells in the plates and incubated for 4 hours. The media was then poured off from the wells of the plates leaving cells alone attached to the surface. Measured 50 µl of DMSO was added to solubilize the formazan crystal formed by viable cells. Absorbance was then read on a scanning multi-well spectrophotometer at 562 nm [10]. Absorbance values higher than the control cells indicated an increase in the rate of cell proliferation and vice versa [11]. The percentage viability was evaluated by determining absorbance with the corresponding chemical concentrations. Linear regression analysis at 95% confidence limits and R^2 were used to define dose-response curves of percentage viability of cells against concentration. Percentage of cell viability was calculated using **Equation 2** and **Equation 3**.

\[
\text{% cell viability} = 100 - \text{% cytotoxicity} \quad (2)
\]

\[
\text{% cytotoxicity} = \frac{A-B}{A} \times 100 \quad (3)
\]

Where A is the optical density of control and B is optical density of test drug.
Data was analyzed to obtain the minimum inhibitory concentration (IC$_{50}$) and median cytotoxic concentration (CC$_{50}$) of the extracts on cancer and Vero cells, respectively [12]. The selectivity index (SI) was calculated as the ratio of CC$_{50}$ to IC$_{50}$ [7, 13].

**Determination of total phenolic content and antioxidant activity**

The TPC of the extracts were determined using Folin-Ciocalteu reagent as described by previous authors [14, 15]. Briefly, 0.5 ml of the extract dissolved in 1 mg/L of methanol in falcon tubes. Gallic acid solutions of 0, 20, 40, 80 and 100 µg/ml were also added into the tubes in methanol : water (50 : 50 v/v) were mixed with 0.5 ml of Folin-Ciocalteu reagent diluted 10-fold in distilled water in falcon tubes and allowed to stand at room temperature for 5 minutes. Exactly 1.5 ml of sodium carbonate (20 g in 100 ml of distilled water) solution was then added, followed by 8.5 ml of distilled water. After 90 minutes, the absorbance was measured using UV-1900 UV Vis Spectrophotometer (Shimadzu Corporation, Japan) at 755 nm using Gallic acid as the standard solution [16].

Antioxidant activity was assessed using DPPH radical scavenging assay as described by Awah and Verla [14]. Briefly, 8.5 ml of methanol was added to 0.1 g of the extracts. From these, 200 µg/ml was made by transferring 0.167 ml of sample stock solutions in different falcon tubes and the volume made up to 10 ml. The solutions were then mixed with 1 ml of 0.1 mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 25 minutes. Blank solutions were prepared with 1 ml of methanol while the negative control was 1 ml of 0.1mM DPPH solution in 2 ml of methanol. Thereafter, the absorbance of the assay mixtures was measured at 517 nm using a UV visible spectrophotometer to measure the decolourization to yellow diphenylpicrylhydrazine. DPPH radical inhibition was calculated using Equation 4.

\[
\% \text{ inhibition} = \left( \frac{A_o - A_s}{A_o} \right) \times 100
\]

Where $A_o$ = the average absorbance of blank (untreated cells) and $A_s$ = absorbance of the sample (treated cells).

**Characterization of compounds in R. officinalis methanolic leaf extract**

The functional groups in the extract fractions were analyzed by Fourier Transform Infrared Spectroscopy (FTIR) using a Shimadzu FTIR spectrometer (Nicolet NEXUS 470, Thermo Scientific, USA). Aliquots (0.1 g) of the fractions were dissolved in 10 ml of methanol. Exactly 0.6 ml of the sample solution was poured on Attenuated Total Reflection (ATR) crystal and the spectra were read at 4500 to 400 cm$^{-1}$. The frequencies of the different components were recorded. The resolution was 4 cm$^{-1}$ for 20 scans on each sample [17, 18]. The analysis was repeated twice for spectra confirmation.
Solid phase extraction and clean up was done for the methanol and ethyl acetate fractions. The end-capped C18 cartridge of sorbent mass, 500 mg; particle size, 50 µm; pore diameter, 48Å; surface area, 526 m²/g was conditioned with 5 ml of 10% methanol in acidified water. Measured 20 ml of each fraction solution was loaded into a C-18 (Supelco, Sigma-Aldrich Germany) column and allowed to flow under gravity. The co-extracted substances were eluted from the sorbent with 100 ml of aqueous acetic acid (2% v/v). The column was dried using a pressure pump in the vacuum manifold for 5 minutes and total retained phenols were eluted with 1.2 ml of 0.1% formic acid acidified methanol [19]. Purified extracts were filtered through a 0.1 µm filter prior to liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analyses [20].

LC-MS/MS was used to identify compounds in the clean-up fractions. The auto-sampler LC system (Finnigan, Thermo Electron Corporation, USA) was coupled to an MS detector (Agilent Technologies, 6420 Triple Quad, USA). Sample solutions of 5 µL were injected into C-18 reverse phase column (Poroshell 120 EC-C18 3 × 50 mm, 2.7 µm, USA) at 40 °C. Data acquisition software was for 6400 Series Triple Quadrupole (Version B.08.00, Qualitative analysis software Version B.07.00 Service Pack 1). Solvent A was made of a mixture of 0.1% formic acid in water and 0.1% ammonium formate in water. It was made by adding 1 ml of formic acid to 1000 ml of water and then a solution of 1.0 g of ammonium formate dissolved in 1000 ml of deionized water and the two solutions were mixed to form solvent A. Solvent B was made of 0.1% formic acid in methanol which was made by adding 0.6 ml formic acid to 600 ml of methanol.

The elution was conducted at a column flow rate of 0.5 ml/min, the pressure of 350 bars, a column temperature of 40 °C at gradient elution for 35 minutes [21]. From 0 to 0.5 minutes, elution was 95% solvent A and 5% solvent B, 0.5-12 minutes was 58% A and 42% B, at 12-15 minutes was 40% A and 60% B, 15-20 minutes was 5% A and 95% B, 20-25 minutes was 5% A and 95% B, 25-25.5 minutes was 90% A and 10% B and then 25-35 min was 95% A and 5% B. The eluent was monitored at Electron spray ionization connected to an ion trap MS (ESI-MS) under negative ion mode at full scan mode of 55-500 m/z [22]. Identification of the compounds was based on retention time in reversed-phase LC and MS spectral features [21].

Statistical analysis

Experiments were done in triplicate and data presented as means ± standard deviations. ANOVA was used to establish any significant differences between extracts and controls. Correlations between antioxidant activity and antiproliferative activity were established using Pearson’s correlation coefficient. Analyses were
performed at $P < 0.05$ using Minitab statistical software (Release 17, Minitab Inc., USA).

**Results and discussion**

**Percentage yield**

The yield of the different extracts, obtained as the percentages of initial mass of the sample macerated is shown in **Table 1**.

**Table 1. Organic extract yield of *R. officinalis* leaves**

| Solvent          | Yield (g) | Percentage yield |
|------------------|-----------|------------------|
| Methanol         | 81.210    | 54.14            |
| Ethyl acetate    | 65.115    | 43.41            |
| Dichloromethane  | 58.005    | 38.67            |
| Hexane           | 32.025    | 21.35            |

Methanol gave the highest yield (54.14%) while n-hexane gave the least yield (21.35%). This is could be due to the differences in polarity as methanol being the most polar gave the highest yield. It could be because it extracted many compounds from the leaves. Differences in solvent polarities used for extraction is known to play a key role in increasing the solubility of phytochemical compounds [23, 24]. Further, differences in the structure of phytochemical compounds also determine their solubility in solvents of different polarities [25]. Indeed, the four solvents used had different polarities arranged as hexane < DCM < ethyl acetate < methanol. Therefore, the results of the current study confirmed the effect of varying solvent polarities on the yield of plant extracts and confirmed the richness of *R. officinalis* leaves in polar phytochemicals. The results obtained are consistent with those of Widyawati et al. [26] who assessed the effects of solvent polarity on the phytochemical yields from *Pluchea indica* leaf extracts.

**Antiproliferative activity of *R. officinalis* leaf extracts and fractions**

The anticancer activity was determined for both crude solvent extracts and fractionated methanol extract. The minimum inhibitory concentrations ($\mu$g/ml) required to give 50% of cell death (IC$_{50}$) by the crude extracts and positive control (doxorubicin) on the prostate, colorectal and cervical cancer cells are shown in **Table 2**. Doxorubicin showed the highest activity on all cancer cell lines compared to the plant extracts ($P < 0.05$). This was evidenced by its very low IC$_{50}$ values (4.36 ± 0.22, 6.39 ± 0.47 and 3.64 ± 0.33 µg/ml for prostate, colorectal and cervical cancer cell lines) compared to the plant extracts.

**Table 2. IC$_{50}$ values (µg/ml) of *R. officinalis* leaf extracts against prostate, colorectal and cervical cancer cells**

| Extract          | DU145  | CT26  | HeLa 229 |
|------------------|--------|-------|---------|
| Methanol         | 147.38 ± 0.53 | 301.99 ± 0.53 | 432.47 ± 0.41 |
| Ethyl acetate    | 182.48 ± 0.50 | 460.08 ± 0.14 | 522.80 ± 1.06 |
| DCM              | 1459.10 ± 0.86 | 928.57 ± 0.49 | 931.63 ± 1.19 |
| Hexane           | Not active | 1104.04 ± 0.06 | 1001.10 ± 0.06 |
| Doxorubicin      | 4.36 ± 0.22 | 6.39 ± 0.47 | 3.64 ± 0.33 |

For results of antiproliferative activity, IC$_{50}$ < 10 µg/ml is considered potentially very toxic; IC$_{50}$
between 10 and 100 µg/ml is potentially toxic; IC\textsubscript{50} between 100 and 1000 µg/ml is potentially harmful and IC\textsubscript{50} > 1000 µg/ml is potentially non-toxic [27]. As shown in Table 2, the methanol extracts were highly toxic on all the cancer cell lines studied compared to other extracts. This is because it showed the least IC\textsubscript{50} values which means, only a small concentration of the extract is required to reduce the number of cancer cells by 50%. Ethyl acetate extract was the second most active, followed by DCM extracts and then finally hexane extracts. This order was also recorded for the TPCs as well as the antioxidant activity of the extracts. This shows that the phenols responsible for the antioxidant activity as well as cytotoxicity of these cancer cells are polar. Correlations between antioxidant activity of the crude extract and antiproliferative activity were established using Pearson’s correlation coefficient. It was found that the antioxidant activity is positively correlated with the antiproliferative activity of the crude extracts against cervical and colorectal cancer cell lines. However, there was a negative correlation for prostate cancer cell lines. The correlation was not statistically significant in all cancer cell lines (\(P > 0.05\)).

Previous studies reported that \textit{R. officinalis} extracts (6.25-50 µg/ml) inhibited viability of DU145 and PC3 prostate cancer cells with IC\textsubscript{50} of about 8.82 µg/ml [28]. The extracts were also effective against colon cancer cell lines: HT-29, HCT116, W480, and HGUE-C-1 for doses between 1.5 to 100 µg/ml with IC\textsubscript{50} between 16.2 and 25 µg/ml [29-33]. For For HeLa (cervical adenocarcinoma), inhibition was at 1.56-400 µg/ml with IC\textsubscript{50} between 10.02 and 23.31 µg/ml [34, 35].

The mechanism of anticancer activity of \textit{R. officinalis} extracts is not clear though. Many studies attributed its antiproliferative activity to enhanced apoptosis and cell death [36]. Increased poly Adenosine diphosphate ribose polymerase (PARP) cleavage, an indicator of enhanced apoptosis was reported for colon, pancreas, breast and lung cancer cell lines [37]. Rosemary extract also increased nitric oxide production and tumor necrosis factor production in pancreatic and liver cancer cells [38, 39], indicating enhanced cell death capabilities and nitric oxide-induced apoptosis. For ovarian cancer cells, enhanced apoptosis was associated with increased gene expression of mitochondrial-regulated apoptosis proteins cytochrome c [40]. These proteins are in the electron transport chain, and along with heat shock protein 70 (hsp70) involved in protein folding protects the cell from heat stress and toxic chemicals. Other mechanisms of apoptosis by Rosemary extracts include enhanced protein expression of pro-apoptotic Bax and cleaved-caspase 3 [32, 41], increased expression of binding immunoglobulin protein (BiP) and enhancer-binding protein homologous proteins (CHOP) which induce endoplasmic reticular stress [33, 41], and the unfolded protein response in prostate and colon
cancer cells [31, 33, 41, 42]. Rosemary extracts have also been reported to exert antioxidant effects in colon, breast and leukemia cell lines, protecting cells from oxidative DNA damage [37].

The results of antiproliferative activity of the fractions from methanolic extract of *R. officinalis* leaves are shown in Table 3. Ethyl acetate fraction showed the highest anticancer activity with IC$_{50}$ of 6.39 ± 0.26, 261.31 ± 0.27 and 119.34 ± 0.38 µg/ml for DU145, CT26 and HeLa 229 cancer cells, respectively. On the other hand, hexane fraction had no activity against CT26 and HeLa 229 cells and had an IC$_{50}$ of 1019.26 ± 0.28 µg/ml for DU145 cells.

### Table 3. IC$_{50}$ values (µg/ml) of the fractions of methanolic extract of *R. officinalis* on the cancer cells

| Fraction      | DU145      | CT26       | HeLa 229  |
|---------------|------------|------------|-----------|
| Methanolic    | 28.28 ± 0.49 | 272.32 ± 0.56 | 385.43 ± 0.52 |
| Ethyl acetate | 8.54 ± 0.47 | 196.02 ± 0.03 | 181.47 ± 0.50 |
| DCM           | 812.49 ± 0.50 | 773.41 ± 0.35 | 569.30 ± 0.58 |
| Hexane        | Not active | 972.26 ± 0.44 | 902.69 ± 0.60 |
| Methanol SPE  | 488.90 ± 1.01 | 521.29 ± 0.50 | 578.74 ± 0.65 |
| Ethyl acetate SPE | 429.30 ± 0.26 | 512.02 ± 0.04 | 550.75 ± 0.53 |
| Doxorubicin   | 4.36 ± 0.22 | 6.39 ± 0.47 | 3.64 ± 0.33 |

SPE: solid phase extract.

The CC$_{50}$ of the extracts and the fractions were determined (Table 4).

### Table 4. CC$_{50}$ values of the tested *R. officinalis* leaf extracts and fractions on Vero cells

| Extract/Fraction | CC$_{50}$ (µg/ml) |
|------------------|------------------|
| Methanol extract | 468.55 ± 0.51    |
| Ethyl acetate extract | 599.27 ± 0.24    |
| DCM extract      | 1253.00 ± 0.62   |
| Hexane extract   | Not applicable   |
| Ethyl acetate fraction | 401.09 ± 0.08    |
| Methanol fraction | 378.38 ± 0.55    |
| DCM fraction     | 1644.64 ± 0.58   |
| Hexane fraction  | Not applicable   |
| Methanol SPE     | 1897.12 ± 0.11   |
| Ethyl acetate SPE | 1841.27 ± 0.47   |
| Doxorubicin      | 6.36 ± 0.45      |

The methanolic fraction of *R. officinalis* showed the least CC$_{50}$ value of 378.38 µg/ml which is potentially harmful while the methanolic solid phase extract showed the highest CC$_{50}$ value of 1897.12 ± 0.11 µg/ml which is potentially non-toxic. The results obtained showed that all the extracts under investigation were less toxic to normal Vero cells, compared to the positive control (doxorubicin) with CC$_{50}$ = 6.36 ± 0.45 µg/ml which is potentially very toxic.

The fractions were comparatively more cytotoxic than the corresponding crude extracts while the solid phase extracts were less cytotoxic when compared to the crude extracts. This could be attributed to greater activity of the polyphenols than in crude extracts where they had interferences [43]. It was observed that the isolates obtained through solid phase extraction showed lower toxicity than the crude extracts and fractions. This could be due to synergistic effects in the crude extracts [30]. Among the fractions, ethyl acetate fraction showed better activity on
the cells than the methanol fraction. This could be due to the fact that ethyl acetate solvent was passed through the column before methanol and it had extracted most of the active compounds from the plant extracts. This was still carried on to the solid phase extracted isolates where it was observed that ethyl acetate isolates showed higher activity ($p < 0.05$).

To further understand the cytotoxicity of the extracts when used for cancer therapy, the selectivity indices were calculated (Table 5). The selectivity index (SI) is the ability of an extract to inhibit the growth of cancer cells more than it does to the normal cells. An extract with the SI $> 3$ is considered to be highly selective and has the potential to be used in the management cancer [13]. Selectivity is the most important feature of an effective anticancer drug and a clear understanding of how much selectivity a new drug should have to be clinically effective is essential [13].

Table 5. Selectivity indices of *R. officinalis* leaf extracts and fractions

| Extract/fraction       | DU145 | CT26 | HeLa 229 |
|------------------------|-------|------|----------|
| Methanolic extract     | 3.18  | 1.55 | 1.08     |
| Ethyl acetate extract  | 3.28  | 1.30 | 1.15     |
| DCM extract            | 0.86  | 1.08 | 1.07     |
| Hexane extract         | NA    | NA   | NA       |
| Methanolic fraction    | 14.18 | 1.47 | 1.04     |
| Ethyl acetate fraction | 44.31 | 1.93 | 2.09     |
| DCM fraction           | 2.02  | 2.13 | 2.89     |
| Hexane fraction        | NA    | NA   | NA       |
| Methanolic SPE         | 3.88  | 3.64 | 3.28     |
| Ethyl acetate SPE      | 4.29  | 3.60 | 3.34     |
| Doxorubicin            | 1.459 | 0.995| 1.747    |

SPE: Solid phase extract, NA: Not applicable

Solid phase extracted clean ups had the highest selectivity indices since they showed selectivity on all cells, followed by the ethyl acetate and methanolic fractions and then the crude extracts then the positive control (doxorubicin). The results showed that doxorubicin was not selective on Vero cells as its selectivity indices were quite lower than 3 [13]. Selective cytotoxicity is a pivotal requirement for anticancer drugs.

**Total phenolic content and antioxidant activity of the extracts**

The TPC of the extracts were determined using the Folin-Ciocalteau method. Folin-Ciocalteau reagent consists of a mixture of sodium molybdate, sodium tungstate and other reagents which when added to plant extracts react with phenolic compounds to produce a solution of a blue complex which absorbs at 760 nm. The assay relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes [44, 45]. A calibration curve (Figure 1) was prepared for the quantitative analysis and the linearity for gallic acid standard was established from the range of 1 µg/ml to 100 µg/ml which was fitted on the line $y = 0.0025x$.  

$$y = 0.0025x$$
The methanol crude extract gave the highest TPC of 476.8 ± 0.69 µg/ml (Table 6). Methanol is a polar protic solvent [23, 46] and thus, it extracted more polyphenols which are inherently polar and their solubility is through hydrogen bond formation [47]. Further, ANOVA test showed that there were significant differences ($p < 0.05$) among the mean TPC of the different solvent extracts. From the results of the antioxidant activity assay (Figure 2), the IC$_{50}$ of methanolic extract (5.39 ± 0.09 mg/ml) was the lowest as compared to 0.06 ± 0.01 mg/ml for ascorbic acid (control). This is because most phenolic compounds responsible for antioxidant activity have polar functional groups which are easily dissolved in polar protic solvents like methanol [26]. The antioxidant activity of plant phenolic compounds is attributed to their redox properties which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers and metal chelators [48]. The DPPH test measures the hydrogen atom or electron donating capacity of extracts to the stable radical DPPH formed in solution [49].

Table 6. Total phenolic content of *R. officinalis* leaf extracts

| Extraction solvent | Total phenolic content (µg/ml GAE) |
|--------------------|----------------------------------|
| Methanol           | 476.8 ± 0.69                     |
| Ethyl acetate      | 74.80 ± 0.80                     |
| Dichloromethane    | 37.47 ± 0.92                     |
| $n$-hexane         | 21.33 ± 0.83                     |

GAE: Gallic acid equivalent

In a study which used *R. officinalis* from different regions of Algeria, Fellah et al. [50] reported TPC ranging from 58.26 ± 0.31 to 114.10 ± 0.15 mg GAE/g dry weight.

![Figure 1](image1.png)  
*Figure 1.* Calibration curve for TPC using Gallic acid standard.

![Figure 2](image2.png)  
*Figure 2.* Antioxidant activity of *R. officinalis* leaf extracts

Similarly, antioxidant activity with IC$_{50}$ values of 8.6 ± 0.5 to 19.4 ± 1.5 µg/ml was reported for *R. officinalis* leaf extracts by Garbarino et al. [51]. Further, Bourhia et al. [52] reported TPC of 146.63, 92.39, 83.27 and 74.15 µg GAE/mg for *R. officinalis* harvested from El Jadida, Taounate,
Beni Mellal and Marrakesh regions of Algeria, respectively. The plants exhibited antioxidant activity with IC$_{50}$ values of 0.302, 0.258, 0.236 and 0.176 mg/ml, respectively.

The antioxidant properties of rosemary have been attributed to its richness in isoprenoid quinones, which act as chain terminators of free radicals and as chelators of reactive oxygen species [53, 54]. Further, compounds such as rosmarinic acid and hesperidin found in rosemary extracts in this study have been cited in the literature as important free radical scavengers [55, 56].

**Characterization of compounds in R. officinalis methanolic leaf extracts**

In the FT-IR spectrum (Figure 3), the intense absorption at 3400 cm$^{-1}$ was due to stretching of phenolic groups present in the extracts. The band at 2900 cm$^{-1}$ was due to stretching of hydroxyl groups like alcohols and water while the absorption at 2800 cm$^{-1}$ could have been due to a C-H group stretching of sp$^3$ hybridized (R$_3$C-H) portion. Absorption at 1700 cm$^{-1}$ is due to stretching of C=O group. The band at 1550 cm$^{-1}$ is due to C=C bonds, typical of aromatic compounds (containing a benzene ring). Absorption at 1400 cm$^{-1}$ was due to asymmetric in-plane bending of –CH$_3$ while at 1350 cm$^{-1}$, the absorption was due to symmetric in-plane bending of –CH$_3$. The stretch at 1250 cm$^{-1}$ is due to nitro groups (-NO$_2$). The absorption at 1100 cm$^{-1}$ was due to C-O stretching vibration. The weak bands at 1000 cm$^{-1}$ and 900 cm$^{-1}$ could be due to C-H bending and terminal C=CH$_2$ groups respectively. These assignments are based on previous studies on phenolic compounds in plants [17, 57, 58]. These confirmed the presence of phenolic compounds in the extract. The various functional groups observed in the extracts reflected the biochemical profile of the leaf extract which could be responsible for the various medicinal properties of this plant leaf, including antiproliferative activity. Phytochemicals such as phenolics, carotenoids, terpenoids and alkaloids from plants have been reported to be key actors in cancer therapy [1, 6, 59].

LC-MS/MS qualitative analysis afforded the identification of 32 compounds in *R. officinalis* methanolic leaf extract (Table 8). These compounds included polyphenols (such as gallic acid, rosmanol, rosmarinic acid), flavonoids, terpenoids and alkaloids. The standards used for quality control in the study (gallic acid and rutin) showed similar LC chromatograms and MS spectra with their corresponding compounds in
the samples. Of the 32 compounds identified, were eight compounds reported for the first time in this plant. These are procyanidin, hydroxyplorentin, cephalin, isoquercetin, latifoliamide, diadzin, hyperin and emetine (Figure 4). Mena et al. [60] reported the presence of (poly)phenolic compounds in leaves. Using ultra-high-performance liquid chromatography-electrospray ionization-mass spectrometry afforded the identification and quantification of 57 compounds, 14 of which were reported for the first time.

Table 8. Compounds identified in *R. officinalis* leaf extract

| Peak | Rt (s) | m/z | MF | Fragments (CE) | Compound |
|------|--------|-----|----|----------------|----------|
| 1    | 2.060  | 191.1 | C\(_{7}\)H\(_{12}\)O\(_{6}\) | 127.0 (24), 93.0 (32) | Quinic acid |
| 2    | 2.967  | 163.1 | C\(_{7}\)H\(_{8}\)O\(_{2}\) | 117.1 (40) | Anustoline |
| 3    | 3.082  | 179   | C\(_{9}\)H\(_{8}\)O\(_{4}\) | 135 (10), 134 (20) | Caffeic acid |
| 4    | 3.128  | 235.2 | C\(_{27}\)H\(_{30}\)O\(_{16}\) | 86.1 (16), 58.2 (36) | Rutin |
| 5    | 8.306  | 220.1 | C\(_{30}\)H\(_{26}\)O\(_{13}\) | 56.1 (52) | Procyanidin |
| 6    | 8.466  | 304.2 | C\(_{15}\)H\(_{15}\)O\(_{6}\) | 182.1 (16), 82(48) | Hydroxyphlorentin |
| 7    | 8.480  | 261.2 | C\(_{15}\)H\(_{14}\)O\(_{6}\) | 182.1 (16), 82(48) | Hydroxyphlorentin |
| 8    | 9.790  | 359.1 | C\(_{18}\)H\(_{16}\)O\(_{9}\) | 123.0 (20), 161.0 (100) | Rosmarinic acid |
| 9    | 13.81  | 313.1 | C\(_{17}\)H\(_{14}\)O\(_{6}\) | 283.0 (32), 298.1 (24) | Cirsimartinit |
| 10   | 13.92  | 345.2 | C\(_{15}\)H\(_{15}\)O\(_{6}\) | 283.2 (100), 301.2 (49) | Rosmanol |
| 11   | 15.14  | 313.1 | C\(_{17}\)H\(_{14}\)O\(_{6}\) | 268.0 (100.0) | Genkwanin |
| 12   | 15.57  | 487.3 | C\(_{30}\)H\(_{48}\)O\(_{5}\) | 65.1(48), 65.1(44) | Cephalin |
| 13   | 16.04  | 487.3 | C\(_{30}\)H\(_{48}\)O\(_{5}\) | - | Asiatic acid |
| 14   | 17.405 | 290.1 | C\(_{15}\)H\(_{10}\)O\(_{7}\) | 168(16), 77(60) | Quercetin |
| 15   | 18.466 | 308.1 | C\(_{20}\)H\(_{32}\)O\(_{12}\) | 163.1 (8), 105.1 (24) | Isoquercetin |
| 16   | 18.558 | 208.1 | C\(_{20}\)H\(_{32}\)O\(_{12}\) | 105.1 (24) | Isoquercetin |
| 17   | 18.826 | 244.2 | C\(_{15}\)H\(_{12}\)O\(_{7}\) | 91.1 (36), 86.2 (8) | Diadzin |
| 18   | 21.91  | 471.3 | C\(_{30}\)H\(_{48}\)O\(_{4}\) | - | Benthamic acid |
| 19   | 22.35  | 471.3 | C\(_{30}\)H\(_{48}\)O\(_{4}\) | - | Augustic acid |
| 20   | 25.425 | 195.1 | C\(_{14}\)H\(_{6}\)O\(_{5}\) | 83 (40) | Eillargic acid |
| 21   | 25.116 | 272.2 | C\(_{20}\)H\(_{32}\)O\(_{12}\) | 215.1 (20), 171.1 (40) | Hyperin |
| 22   | 27.15  | 234.1 | C\(_{20}\)H\(_{32}\)O\(_{14}\) | 84.3 (20) | Naringin |
| 27   | 29.179 | 220.1 | C\(_{20}\)H\(_{32}\)O\(_{12}\) | 84.1 (16) | Hesperiden |
| 28   | 29.234 | 153   | C\(_{15}\)H\(_{14}\)O\(_{4}\) | 109 (10), 108 (20) | Gentisic acid |
| 29   | 29.384 | 177.1 | C\(_{15}\)H\(_{14}\)O\(_{4}\) | 98.1 (24), 80.1 (28) | Chlorogenic acid |
| 30   | 29.405 | 136.1 | C\(_{20}\)H\(_{48}\)O\(_{4}\) | 119 (4), 91 (16) | Emetine |
| 31   | 29.719 | 209.2 | C\(_{17}\)H\(_{32}\)O\(_{4}\) | 124.1 (24), 93.1 (32) | Atropine |
The rosemary extract contained 24 flavonoids (mainly flavones), 5 phenolic acids, 24 diterpenoids (carnosic acid, carnosol, and rosmanol derivatives), 1 triterpenoid (betulinic acid) and 3 lignans (medioresinol derivatives). Carnosic acid was reported as the dominant phenolic compound in the extracts [60]. The compounds identified were Medioresinol, p-Coumaric acid, Luteolin-rutinoside, Luteolin-hexoside, Isorhamnetin-3-O-hexoside, 4-hydroxybenzoic acid, Apigenin-7-O-glucoside, Homoplantaginin (Hispidulin 7-glucoside) among others which have been previously identified in this plant [38, 61-64]. Five phenolic acids (a hydroxybenzoic acid, two hydroxycinnamic acids and two rosmarinic acid derivatives) were identified, substantiating previous observations in this species [61, 64].

Some of the compounds identified such as ursolic, rosmarinic and gallic acids were previously reported to have anticancer activity [36, 65]. Thus, the results of this study supports the traditional use of this plant in cancer therapy in Uganda.
Conclusions
The results of this study showed that *R. officinalis* extracts has phenolic compounds with antiproliferative activity against human prostate (DU145), colorectal (CT26) and cervical (HeLa 229) cancer cells. Selectivity of *R. officinalis* leaves in antiproliferative activity followed the order: solid phase extracted clean ups > ethyl acetate and methanolic fractions > crude extracts. Further studies should evaluate the anticancer activity of the extracts on other cancer cell lines because some of the polyphenols could be inactive on the cell lines investigated in this study yet active on the other cell lines that have not been studied. Studies on the anticancer potential of some of the identified unstudied compounds should be taken. The chemical composition and antiproliferative activity of *R. officinalis* roots should be done.

Acknowledgements
The authors would like to thank the management of Directorate of Government Analytical Laboratory, Natural Chemotherapeutics Research Institute, Kampala, Uganda and Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute, Nairobi, Kenya for their analytical support and services rendered. The World Bank and the Inter-University Council of East Africa are acknowledged for the fellowship awarded to Winfred Nassazi through the Africa Center of Excellence II in Phytochemicals, Textiles and Renewable Energy (ACE II PTRE, Credit No. 5798-KE) which led to this communication. Timothy Omara (Department of Chemistry and Biochemistry, Moi University) is greatly acknowledged for his selfless prepublication support and for English proofreading of this article.

References
[1] Omara T, Kiprop AK, Ramkat RC, Cherutoi J, Kagoya S, Nyangena DM, et al. Medicinal Plants Used in Traditional Management of Cancer in Uganda: A Review of Ethnobotanical Surveys, Phytochemistry, and Anticancer Studies. Evid-based Complement Altern Med. 2020; 1-26. doi: 10.1155/2020/3529081.
[2] Akter R, Uddin SJ, Grice ID, Tiralongo E. Cytotoxic activity screening of Bangladeshi medicinal plant extracts. J Nat Med. 2014; 68: 246-252.
[3] Khalighi-Sigaroodi F, Ahvazi M, Hadiakhoondi, A, Taghizadeh M, Yazdani D, Khalighi-Sigaroodi S, Bidel S. Cytotoxicity and antioxidant activity of 23 plant species of Leguminosae family. Iranian J Pharmaceut Res. 2012; 11(1): 295-302.
[4] Omara T, Nassazi W, Omute T, Awath A, Laker F, Kalukusu R, et al. Aflatoxins in Uganda: An Encyclopedic Review of the Etiology, Epidemiology, Detection, Quantification, Exposure Assessment, Reduction, and Control. Int J Microbiol. 2020; 2020(4723612): 1-26. doi: 10.1155/2020/4723612
[5] Nguyen C, Mehaidli A, Baskaran K, Grewal S, Pupulin A, Ruvinov I, Pandey S. Dandelion Root and Lemongrass Extracts Induce Apoptosis, Enhance Chemotherapeutic Efficacy, and Reduce Tumour Xenograft Growth in vivo in Prostate Cancer. Evid-Based Complement Altern Med. 2019; 2019(2951428): 1-12.
[6] Kaur R, Kapoor K, Kaur H. Plants as a source of anticancer agents. J Nat Prod Plant Resour. 2011; 1(1): 119-124.

[7] Majoumouo MS, Tincho MB, Morris T, Hiss DC, Boyom FF, Mandal C. Antiproliferative potential of methanolic and aqueous extracts and their methanolic fractions derived from fruits of Bersama engleriana against a panel of four cancer cell lines. Cogent Biol. 2020; 6(1): 1727636.

[8] Azwanida NN. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med Arom Plants. 2015; 4:3. doi: 10.4172/2167-0412.1000196

[9] Torres RG, Casanova L, Carvalho J, Marcondes MC, Costa SS, Sola-Penna M, Zancan P. Ocimum basilicum but not Ocimum gratissimum present cytotoxic effects on human breast cancer cell line MCF-7, inducing apoptosis and triggering mTOR/Akt/p70S6K pathway. J Bioenerg Biomembr. 2018; 50(2): 93-105.

[10] Nenavath V, Darling CD. Evaluation and comparison of anti-cancer activity of dapagliflozin and canagliflozin in oral cancer cell line : an in vitro study. Int J Basic Clin Pharmacol. 2019; 8(3): 473-477.

[11] Horn S, Pieters R, Bezuidenhout C. A cell viability assay to determine the cytotoxic effects of water contaminated by microbes. South Afr J Sci. 2013; 109(7-8): 01-04.

[12] El-Attar MM, Awad AA, Abdel-Tawab FM, Kamel HA, Ahmad ES, Hassan AI. Assessment of cytotoxic and anticancer activity of Zygophyllum album and Suaeda palastina extracts on human liver cancer cell lines. J Agric Life Sci. 2019; 27(1): 539 - 544.

[13] Njuguna DK, Mbuthia K, Mutuku C, Jepkorir M, Wanjiru J, Mwangangi R, Mwitari P. Phytochemical Composition and In vitro Anti-Proliferative Activity of Oxygonum sinuatum (Meisn.) Dammer on Selected Cancerous Cells. J Complement Altern Med Res. 2018; 6(2): 1-9.

[14] Awah FM, Verla AW. Antioxidant activity, nitric oxide scavenging activity and phenolic contents of Ocimum gratissimum leaf extract. J Med Plants Res. 2010; 4(24): 2479-2487.

[15] Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agric Food Chem. 1998; 46(10): 4113-4117.

[16] Krishnaiah D, Devi T, Sarbatly R, Bono A, Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants. J Med Plants Res. 2009; 3(32): 067-072.

[17] Ashokkumar R, Ramaswamy M. Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. Int J Curr Microbiol Appl Sci. 2014; 3(1): 395-406.

[18] Silva SD, Feliciano RP, Boas LV, Bronze MR. Application of FTIR-ATR to Moscatel dessert wines for prediction of total phenolic and flavonoid contents and antioxidant capacity. Food Chem. 2014; 150: 489-493

[19] Rajauria G. Optimization and validation of reverse phase HPLC method for qualitative and quantitative assessment of polyphenols in seaweed. J Pharmaceut Biomed Anal. 2018; 148: 230-237.

[20] Quatrin A, Pauletto R, Maurer LH, Minuzzi N, Nichelle SM, Carvalho JFC, Emanuelli T. Characterization and quantification of tannins, flavonols, anthocyanins and matrix bound polyphenols from jaboticaba fruit peel: A comparison between Myrciaria trunciflora and M. jaboticaba. J Food Compos Anal. 2019; 78: 59-74.

[21] Zhong L, Wu G, Fang Z, Mark L, Hodgson JM, Clarke MW, Johnson SK. Characterization of polyphenols in Australian sweet lupin (Lupinus
[22] Ren J, Liao L, Shang S, Zheng Y, Sha W, Yuan E. Purification, Characterization, and Bioactivities of Polyphenols from Platycladus orientalis (L.) Franco. J Food Sci. 2019; 84(3): 667-77.

[23] Felhi S, Daoud A, Hajlaoui H, Mnafgui K, Gharsallah N, Kadri A. Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of Ecballium elaterium seeds and peels fruits. J Chem Pharmaceut Sci. 2017; 37(3): 483-492.

[24] Naima R, Oumam M, Hannache H, Sesbou A, Charrier B, Pizzi A, Charrier-El Bouhtoury F. Comparison of the impact of different extraction methods on polyphenols yields and tannins extracted from Moroccan Acacia mollissima barks. Indus Crops Prod. 2015; 70: 245-252.

[25] Felhi S, Baccouch N, Ben Salah H, Smaoui S, Allouche N, Gharsallah N, Kadri A. Nutritional constituents, phytochemical profiles, in vitro antioxidant and antimicrobial properties, and gas chromatography-mass spectrometry analysis of various solvent extracts from grape seeds (Vitis vinifera L.). Food Sci Biotechnol. 2016; 25(6): 1537-1544.

[26] Widyawati PS, Dwi T, Budianta W, Kusuma FA. Difference of solvent polarity to phytochemical content and antioxidant activity of Pluchea indicia Less leaves extracts. Int J Pharmacog Phytochem Res. 2014; 6(4): 850-855.

[27] Hussain AI, Anwar F, Chatha SAS, Jabbar A, Mahboob PS, Nigam, S. Rosmarinus officinalis: antiproliferative, antioxidant and antibacterial activities. Brazilian J Microbiol. 2010; 41(4): 1070-1078.

[28] Yesil-Celiktas O, Sevimli C, Bedir E, Vardar-Sukan, F. Inhibitory effects of rosemary extracts, carnosic acid and rosmarinic acid on the growth of various human cancer cell lines. Plant Food Hum Nutr. 2010; 65: 158-163.

[29] González-Vallinas M, Molina S, Vicente G, Zarza V, Martín-Hernández R, García-Risco MR, et al. Expression of MicroRNA-15b and the Glycosyltransferase GCNT3 Correlates with Antitumor Efficacy of Rosemary Diterpenes in Colon and Pancreatic Cancer. PLoS ONE. 2014; 9: e98556.

[30] Borrás-Linares I, Pérez-Sánchez A, Lozano-Sánchez J, Barrajón-Catalán E, Arráez-Román D, Cifuentes A, et al. A bioguided identification of the active compounds that contribute to the antiproliferative/cytotoxic effects of rosemary extract on colon cancer cells. Food Chem Toxicol. 2015; 80: 215-222.

[31] Valdés A, Sullini G, Ibáñez E, Cifuentes A, García-Cañas V. Rosemary polyphenols induce unfolded protein response and changes in cholesterol metabolism in colon cancer cells. J Funct Foods. 2015; 15: 429-439.

[32] Yan M, Li G, Petiwala SM, Householter E, Johnson JJ. Standardized rosemary (Rosmarinus officinalis) extract induces Nrf2/sestrin-2 pathway in colon cancer cells. J Funct Foods. 2015; 13: 137-147.

[33] Valdés A, García-Cañas V, Koçak E, Simó C, Cifuentes A. Foodomics study on the effects of extracellular production of hydrogen peroxide by rosemary polyphenols on the anti-proliferative activity of rosemary polyphenols against HT-29 cells. Electrophoresis. 2016; 37: 1795-1804.

[34] Berrington D, Lall N. Anticancer Activity of Certain Herbs and Spices on the Cervical Epithelial Carcinoma (HeLa) Cell Line. Evid-Based Complement Alternat Med. 2012; 2012: e564927.

[35] Dilas S, Knez Ž, Cetojevic-Simin D, Tumbas V, Škerget M, Canadanovic-Brunet J, et al. In vitro
antioxidant and antiproliferative activity of three rosemary (Rosmarinus officinalis L.) extract formulations. Int J Food Sci Technol. 2012; 47: 2052-2062.

[36] Moore J, Yousef M, Tsiani E. Anticancer Effects of Rosemary (Rosmarinus officinalis L.) Extract and Rosemary Extract Polyphenols. Nutrients. 2016; 8(11): 731.

[37] Moore J, Megaly M, MacNeil AJ, Klintour P, Tsiani E. Rosemary extract reduces Akt/mTOR/p70S6K activation and inhibits proliferation and survival of A549 human lung cancer cells. Biomed Pharmacotherap. 2016; 83: 725-732.

[38] Kontogianni VG, Tomic G, Nikolik I, Nerantzaki AA, Sayyad N. Phytochemical profile of Rosmarinus officinalis and Salvia officinalis extracts and correlation to their antioxidant and anti-proliferative activity. Food Chem. 2013; 136: 120-129.

[39] Peng CH, Su JD, Chyau CC, Sung TY, Ho SS, Peng CC, Peng HY. Supercritical Fluid Extracts of Rosemary Leaves Exhibit Potent Anti-Inflammation and Anti-Tumor Effects. Biosci Biotechnol Biochem, 2007. 71: p. 2223-2232.

[40] Tai J, Cheung S, Wu M, Hasman D. Antiproliferation effect of Rosemary (Rosmarinus officinalis) on human ovarian cancer cells in vitro. Phytomed. 2012; 19: 436-443.

[41] Petiwala SM, Berhe S, Li G, Puthenveetil AG, Rahman O, Nonn L, et al. Rosemary (Rosmarinus officinalis) Extract Modulates CHOP/GADD153 to Promote Androgen Receptor Degradation and Decreases Xenograft Tumor Growth. PLoS ONE. 2014; 9: e89772.

[42] Valdés A, Artemenko KA, Bergquist J, García-Cañas V, Cifuentes A. Comprehensive Proteomic Study of the Antiproliferative Activity of a Polyphenol-Enriched Rosemary Extract on Colon Cancer Cells Using Nanoliquid Chromatography-Orbitrap MS/MS. J Proteome Res. 2016; 15: 1971-1985.

[43] Eknunwe SI, Thomas MS, Luo X, Wang H, Chen Y, Zhang X, Begonia GB. Potential cancer-fighting Ocimum gratissimum (OG) leaf extracts: increased anti-proliferation activity of partially purified fractions and their spectral fingerprints. Ethnity Dis. 2010; 20: S1-16.

[44] Everette JD, Bryant QM, Green AM, Abbey YA, Wangila GW, Walker RB. Thorough study of reactivity of various compound classes toward the Folin-Ciocalteu reagent. J Agric Food Chem. 2010; 58(14): 8139-44.

[45] Ainsworth E, Gillespie K. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nature Protocol. 2007; 2: 875-877

[46] Omara T, Kagoya S, Openy A, Omute T, Ssebulime S, Kiplagat KM, et al. Antivenin plants used for treatment of snakebites in Uganda: ethnobotanical reports and pharmacological evidences. Trop Med Health. 2020; 48:6. doi: 10.1186/s41182-019-0187-0

[47] Sripad G, Prakash V, Rao MSN. Extratability of polyphenols of sunflower seed in various solvents. J Biosci. 1982; 4(2): 145-152.

[48] Cook NC, Samman S. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr Biochem. 1996; 7(2): 66-76.

[49] Tepe B, Dafarera D, Sokmen A, Sokmen M, Polissiou M. Antimicrobial and antioxidant activities of the essential oil and various extracts of Salvia tomentosa Miller (Lamiaceae). Food Chem. 2005; 90: 333-340.

[50] Felliha O, Bourenane N, Hameurlaine S, Altun M, Gherras N, Zellagui A, et al. Anticancer Activity of Rosmarinus Officinalis Aqueous Extracts from Three Locations in Algeria. World J Environ Biosci. 2014; 7(3): 39-42.
[51] Garbarino JA, Troncoso N, Delpiano P, Carvajal L, Russo A. Antioxidant Activity Analysis for the Selection of Rosmarinus officinalis L. Nat Prod Comm. 2006; 1(12): 1123-1128.

[52] Bourhia M, Laasri FE, Aourik H, Boukhris A, Ullah R, Bari A, Gmouh S. Antioxidant and Antiproliferative Activities of Bioactive Compounds Contained in Rosmarinus officinalis Used in the Mediterranean Diet. Evid-Based Complement Altern Med. 2019; 2019: 1-7.

[53] Hölihan CM, Ho CT, Chang SS. The structure of rosmariquinone-A new antioxidant isolated from Rosmarinus officinalis L. J Am Oil Chem Soc. 1985; 61: 1036-39.

[54] Wu JW, Lee MH, Ho CT, Chan SS. Elucidation of the chemical structures of natural antioxidants isolated from rosemary. J Am Oil Chem Soc. 1982; 59: 339-345.

[55] Souza LC, de Gomes MG, Goes ATR, Del Fabbro L, Filho CB, Boeira SP, Jesse CR. Evidence for the involvement of the serotonergic 5-HT1A receptors in the 2 antidepressant-like effect caused by hesperidin in mice Q13. Prog Neuro-Psychopharmacol Biol Psychiat. 2012; 40:103-109.

[56] Yang SY, Hong CO, Lee GP, Kim CT, Lee WW. The hepatoprotection of caffeic acid and rosmarinic acid, major compounds of Perilla frutescens, against t-BHP-induced oxidative liver damage. Food Chem Toxicol. 2013; 55: 92-99.

[57] Singh P. Infrared Spectroscopy. J Anal Chem. 2016: 1-85.

[58] Meenakshi S, Umayaparvathi S, Arumugam M, Balasubramanian T. In vitro antioxidant properties and FTIR analysis of two seaweeds of Gulf of Mannar. Asian Pac J Trop Biomed. 2012; 1: S66-70.

[59] Graham JG, Quinn, M. L., Fabricant, D. S., & Farnsworth, N. R. Plants used against cancer - an extension of the work of Jonathan Hartwell. J Ethnopharmacol. 2000; 73(3): 347-377.

[60] Mena P, Cirlini M, Tassotti M, Herrlinger KA, Dall'asta C, Del Rio D. Phytochemical Profiling of Flavanoids, Phenolic Acids, Terpenoids, and Volatile Fraction of a Rosemary (Rosmarinus officinalis L.) Extract. Molecules. 2016; 21:1576.

[61] Hossain MB, Rai DK, Brunton NP, Martin-Diana AB, Barry-Ryan AC. Characterization of phenolic composition in lamiaceae spices by LC-ESI-MS/MS. J Agric Food Chem. 2010; 58: 10576-81.

[62] Romo-Vaquero M, Yañez-Gascón MJ, Villalba R, Larrosa M, Fromentin E, Ibarra A, et al. Inhibition of gastric lipase as a mechanism for body weight and plasma lipids reduction in Zucker rats fed a rosemary extract rich in carnosic acid. PLoS ONE. 2012; 7: e39773.

[63] Segura-carretero A, Fernández-gutiérrez A. Comparison of different extraction procedures for the comprehensive characterization of bioactive phenolic compounds in Rosmarinus officinalis by reversed-phase high-performance liquid chromatography with diode array detection coupled to electrospray time. J Food Compos Anal. 2011; 1218: 7682-90.

[64] Pérez-Fons L, Garzón MT, Micol V. Relationship between the antioxidant capacity and effect of rosemary (Rosmarinus officinalis L.) polyphenols on membrane phospholipid order. J Agric Food Chem. 2010; 58:161-171.

[65] Nangia-Makker P, Tait L, Shekhar MP, Palomino E, Hogan V, Piechocki MP, et al. Inhibition of breast tumor growth and angiogenesis by a medicinal herb: Ocimum gratissimum. Int J Cancer. 2007; 121(4): 884-894.