Egypt. J. Chem. Vol. 63, No.10 pp 3779 - 3790

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Saraca indica L. leaves, Phytochemical, *in vitro* Antioxidant and Cytotoxic Activities

Wedian El-Sayed Ashour[1 *], Reda Sayed Mohammed[1]*, Aisha Hussein Abou Zeid[1], Omar M. Sabry[2] and Seham S. El Hawary[2]

[1] *Department of Pharmacognosy, National Research Centre; 33-Elbohouth St, (Former El-Tahrir St.), Dokki – Giza-Egypt. P.O.12622, ID: 60014618*
[2]*Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr el Aini Street, Cairo, 11562, Egypt*

Abstract

Saraca indica L. (*Saraca asoca)* is reported for its important biological benefits in Indian folk medicine. The powdered leaves were extracted with 70% ethanol to obtain total ethanol extract (TEE) that was successively fractionated to get petroleum ether (pet. ether), chloroform($CHCl_3$), ethyl acetate (EtOAc) and methanol extracts. The antioxidant activity of TEE by three methods of assay, as well as, the antioxidant activity of TEE and successive extracts by DPPH method at two dose were done.Total phenolics and total flavonoids content of the plant were determined. The cytotoxic evaluation revealed that EtOAc extract exhibited activity against HCT-116 and MCF-7, Hela & HEPG-2 . GC/MS analysis of the unasponifiable(USF) and saponifiable (SF)fractions of the non-polar extract was carried out. 34 Compounds with phytol, dodecane, BHT and α -amyrin as the major compounds in the USF and 26 compounds with methyl palmitate & Methyl-9, 12,15- octadecatrienoate as major compounds in the SF were identified. Fifteen phenolic compounds were detected in HPLC analysis of EtOAc extract as well as six phenolic compounds were isolated and identified for the first time from the same extract they were; gallic acid, methylgallate, rutin, quercetin -3-*O*-rhamnoside, quercetin and kaempeferol.

Keywords: *Saraca indica* L., Antioxidant, Cytotoxic activities, Total phenolic, Total flavonoids, Unsaponifiable, Saponifiable fractions

Introduction

Saraca indica L is the most ancient tree in India, generally known as *Saraca asoca* or ashoka belonging to family *Caesalpiniaceae*. The useful parts of the plant are the bark, leaves, flowers, and seeds. The ashoka is a rain-forest tree. Its original distribution was in the central areas of the Deccan plateau, as well as the middle section of the Western Ghats in the western coastal zone of the Indian subcontinent [1]. *Saraca indica* is reported to contain glycosides, flavonoids, tannins, and saponins[2]. It has many health benefits and has an important role in Indian cultural traditional medicine. It acts as a key ingredient in various therapies and cures[3, 4] [5]. The dried bark and flower of *Saraca asoca* is given to treat uterine disorders, it has a nourishing effect on the circulatory system, encouraging urine flow, it also posses many pharmacological activities as in jaundice, joint pain, kidney stones, gall stones, paralysis, skin problems and rheumatoid arthritis [6]different extracts of *Saraca indica* showed

*Corresponding author e-mail: redamohammed2015@gmail.com., reda_sayed2008@yahoo.com
EJCHEM use only: Received date here; 28 Jan 2020 revised date here; accepted date here 4 March 2020
DOI: 10.21608/EJCHEM.2019.6778.1566

antimicrobial activity [2] [7] [8] ; it can improve the biological profile of the body(renal and liver)[8,7]. In the current study we aim to prove some of the Indian folk medicinal uses of the plant on a scientific bases as the antioxidant activity through the use of three different methods (DPPH, FRAP assay &ABTS) and the cytotoxic activity by using human cancer cell lines on the different extracts of the plant as well as investigation of the phytochemical contents of the extracts which are responsible for these activities. The current study was done for the first time.

Material and methods

UV/VIS (Ultraviolet and Visible Absorption Spectrometer, Labomed Inc.) for measuring UV spectral data of the isolated compounds, in the range of 200–500 nm in methanol and with different diagnostic shift reagents. NMR (Nuclear Magnetic Resonance Spectrophotometer, Bruker, 400 MHz for ^{1}H-NMR).Thermo Sientific, ISQ Single Quadrupole MS ionization enegry 70 ev for measurment of EI/MS(USA). Diaion HP-20, Column chromatography (CC,120cm length, 10cm diameter),Sephadex LH-20 (Pharmazia) for purification of the isolated compounds. PC using Whatman No. 1 and 3 MM with solvent systems: 15% HOAc -H_2O; BAW (n-BuOH: HOAc : H_2O 4:1:5, upper layer).Complete acid hydrolysis for *O*-glycosides was carried out & followed by Co-chromatograph with authentic samples to identify the aglycone and sugar moieties. Agilent 1260 series(Agilent Technologies, Waldbronn, Germany) with a diode array detector. The separation was carried out using the Kromasil C_{18} column (4.6 mm x 250 mm i.d., 5 µm) was used in HPLC determination of phenolic compounds. Source of solvents used for plant extraction were purchased from Algomhorea company for chemical compounds (Cairo, Egypt). Materials used for the determination of total phenolic (TP) and total flavonoid (TF) compounds were as follows: Folin–Ciocalteu reagent, gallic acid, quercetin, $NaNO_2$, Na_2CO_3, $AlCl_3$ (Merck, Germany). Material used for antioxidant study: 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, Trolox, ferrozine, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonicacid(ABTS),TPTZ (2, 4, 6-tripyridyl-s-triazine) -$FeCl_3$•$6H_2O$ solution. All chemical$_s$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All solvents were of analytical grade. Materials for cytotoxic activity four human cancer cell lines; Breast, Colon, Liver and Cevix (MCF-7, HCT-116, HEPG-2, and HeLa) were obtained from the National Organization for Drug Control and Research(NODCAR).

Plant material

The fresh leaves of *Saraca indica* L. were collected from Al -Orman Garden(Giza, Egypt). The plant was identified by Mrs. Terase Labib, plant taxonomist of Al-Orman Garden. A voucher specimen has been deposited in the Herbarium of Faculty of Pharmacy, Pharmacognosy Dept. On 28/10/2015. PCG: 233. The plant was air-dried, powdered and kept in well closed dark colored containers in a cold place.

Preparation of extracts

One Kg of the powdered plant under investigation was extracted by petroleum ether (60-80°C), chloroform, ethyl acetate and methanol 70 %. The solvents were evaporated to dryness under reduced pressure at 55°C. *S. indica* L. yielded (40 g) (4%) PE (31 g) ((3.1%) $CHCl_3$, (75g) (10%) EtOAc and (90g) (9%) MeOH extracts

Preparation Total ethanol extracts (TEE crude extracts)

200 g of powdered leaves of *Saraca indica* L. was extracted with 70% ethanol in a continuous extraction apparatus and evaporation of the extract under vacuum give 56 g of *crude extracts* TEE

Biological study

Antioxidant activity

Determination of free radical scavenging activity (DPPH)

The free radical scavenging activity of different extracts and fractions is measured by DPPH• using the method of Yamaguchi et al [9]. Overall, 1ml of DPPH• solution (0.1mmol/l DPPH• in methanol) was added to 3ml of each concentration of samples and standard material (Vit. C). The mixture was shaken vigorously and allowed to stand at room temperature for 30min. Then the absorbance was measured at 517nm in a spectrophotometer. The control sample was prepared with the same procedure. The DPPH• radical concentration in the reaction medium was calculated from the following equation:

Antioxidant activity (%) = [(Abs. control – Abs. Sample)] / (Abs. control)] × 100

Determination of ferric reducing antioxidant power (FRAP) [10]

The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 m M $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution,

and 2.5 ml $FeCl_3 \cdot 6H_2O$ solution and then warmed at 37 °C before using. The extract was allowed to react with 2850 µl of the FRAP solution for 30 min in the dark condition. Readings of the colour product (ferrous tripyridyltriazine complex) were then taken at 593 nm. Results are expressed in µmol Trolox / g dry matter. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

Determination of ABTS radical scavenging activity [11]

The stock solutions included, 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 hr at room temperature in the dark. The solution was then diluted by mixing 1 ml $ABTS.^+$ solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. $ABTS.^+$ Solution was freshly prepared for each assay. Plant extract (0.5 ml) was allowed to react with 3 ml of the ABTS. $^+$ solution and the absorbance were taken at 734 nm after 7 min using the spectrophotometer. The $ABTS.^+$ scavenging capacity of the extract as percentage inhibition calculated as ABTS radical scavenging activity using the following equation. (%) = [(Abs. Control – Abs. Sample)] / (Abs. Control)] × 100

Where Abs. Control is the absorbance of ABTS radical + methanol; Abs. the sample is the absorbance of ABTS radical + sample extract.

Each of the above assays was carried out in triplicate.

Determinations of DPPH, FRAP and ABTS radical scavenging activity of different extracts at two dose levels(0.01g,0.5g)

Cytotoxic evaluation

Cytotoxic effect of the TEE, pet. ether, $CHCl_3$, EtOAc and methanol fraction of the leaves of *saraca indica* L. as well as doxorubicin (reference drug) were accomplished on four human cancer cell lines HepG2, MCF-7, HCT-116 and HeLa. They were obtained from the National Organization for Drug Control and Research(NODCAR),MTT assay was used to test the cytotoxic activities of these extracts [12]. Data were subjected to paired samples SPSS Statistical Software Package (version 8.0). $P<0.005$ was regarded as significant. Also, probit analysis was carried for IC_{50} determination according to the reported method.

Principle of the assay:

SRB Cell survival assay:

Cell survival was determined using Sulpho Rhodamine-B (SRB) method [12]

Potential cytotoxicity of the extracts against human tumor cell lines was determined as follows:

Cells were plated in 96-multi-well plate (10^4 cells/ well) for 24 h. Before treatment with the test sample to allow attachment of the cell to the plate wall. Different concentrations of the sample under test (0.01, 0.1, 1, 10 & 100 µg/ml in DMSO) were added to the cell monolayer. The assay was conducted in triplicates for each dose. Monolayer cells were incubated with the test solutions for 48 h. at 37^{o}C and in an atmosphere of 5% CO_2.After 48 h., cells were fixed, washed and stained with sulforhodamine B (SRB) stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris-EDTA buffer.Color intensity was measured in an ELISA reader. The relation between the surviving fraction and drug conc. was plotted to get the survival curve of each tumor cell line after treatment with the specified sample. The potency was compared with reference drugs; doxorubicin (DOX).The IC_{50} values were calculated using sigmoidal concentration-response curve fitting models (Sigma plot software program).

Phytochemical study

phytochemical screening

The TEE, pet ether, $CHCl_3$, EtOAc and methanol extracts were subjected to phytochemical screening tests to detect chemical classes in different extracts

Determination of total phenolic(TP) and total flavonoid(TF) content

TP and TF assays were conducted using the modified method of Marinova, et al [13], where in 0.5 g of powdered leaves was extracted using 50 ml of 80% aqueous methanol in an ultrasonic bath for 20 min. Then the mixture was centrifuged for 5 min at 14000 rpm. The supernatant was collected and used for TP and TF quantification.

Determination of total phenolic content

Total phenolics were assayed using the Folin–Ciocalteu assay. A volume of 1ml of the extract or standard solution of gallic acid (20, 40, 60, 80, 100 mg/l) was used; 1ml of Folin–Ciocalteu's phenol reagent was added to it, and the mixture was mixed and shaken. After 5 min, 10 ml of 7% sodium carbonate was added; the mixture was then completed to 25 ml with distilled water, mixed, and allowed to stand at room temperature for 90 min. The absorbance against the prepared reagent blank was determined at 750nm using a spectrophotometer (Jasco V630 spectrophotometer, Guangdong, China (Mainland)). The TP content was expressed as mg gallic acid equivalents/g extract. The results were

derived from a calibration curve Where: y = 0.0531x + 0.0003, R2 = 0.9951Gallic acid equivalents/g extract[14].

Determination of total flavonoid content

TF was determined using the $AlCl_3$ colourimetric method [13]. A volume of 1 ml of plant extract or standard solution of quercetin (20, 40, 60, 80, 100mg/l) was added to 4 ml of distilled water in a 10 ml volumetric flask. A volume of 0.3 ml of 5% $NaNO_2$ was added after 5 min, and 0.3ml of 10% $AlCl_3$ was added and left for 6 min. Thereafter, 2ml of 1mol/l NaOH was added. The mixture was diluted to 10ml with distilled water. The absorbance of the solution was measured at 510 nm. The results were expressed as mg quercetin equivalents (Qu)/g, and all samples were analyzed in triplicate. The results were derived from the calibration curve.

Y = 0.0291×−0.0397, R2 = 0.9904. and expressed in quercetin equivalents / gram extract [14]

Investigation of the non -polar extract Saponification of the non- polar extract and preparation of the fatty acid methyl esters

Five gm of non- polar extracts**(**pet. ether + $CHCl_3$) of *Saraca indica* L leaves were subjected to saponification and preparation of fatty acid methyl esters according to reported method [15] to get 2.5 g of te unsaponifiable fraction (USF)and 1.5 g of fatty acid fraction the latter subjected to methylation to obtain fatty acids methyl ester fraction (FAMEF).

GC/MS analysis of the USF and FAMEF

GC/MS analysis of the USF and FAMEF was carried out using the following conditions: Capillary column of Thermo Scientific TR-5MS (5%phenyl polysilphenylene siloxane), 30m length, 0.25 mm, internal diameter, and 0.25 µm thickness, The identification of the compounds was accomplished by comparing their retention times and mass spectral data with those of the library and published data [16] . Quantitative determinations were carried out based on peak area measurements.

High-performance liquid chromatography (HPLC) analysis

HPLC analysis of ethyl acetate extract was carried out using an Agilent 1260 series(Agilent Technologies, Waldbronn, Germany) with diode array detector. The separation was carried out using a Kromasil C_{18} column (4.6 mm x 250 mm i.d., 5 µm).The mobile phase consisted of water (A) and 0.05% tri fluoroacetic acid in acetonitrile (B) at a flow rate 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (85% A) and 15-16 min (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 µl for each of the sample solutions. The column temperature was maintained at 35 °C. This analysis enabled the characterization of phenolic compounds based on their retention time and UV spectra.

Column chromatographic isolation and purification of EtOAc extract content

20 gm of the EtOAc extract was fractionated on Diaion HP-20, eluted with 100% water and thereafter the polarity was decreased by adding methanol 25, 50, 75, and finally 100% to obtain five substantial fractions (fractions A–E).Fraction B and C were subjected to different chromatographic techniques including 3MM preparative paper chromatography and repeated Sephadex LH-20 column using eluents of different polarities this led to the isolation and purification of six compounds 1, 2, 3, 4,5& 6 . The isolated compounds were structurally elucidated through R_f values, colour reactions, chemical investigations (complete acid hydrolysis) and spectral investigations (UV, NMR, and MS) [17] [18] [19]

Results

The results of the antioxidant screening by three different methods revealed that the % scavenging activities were; DPPH: 154.7 µmol TE/g; FRAP assay:141.29 µmol TE/g and ABTS:148.32 µmol TE/g (TE: trolox equivalent), (Table 1).Investigation of the antioxidant activity of two doses level of different extracts of *Saracca indica* L. by DPPH assay revealed that there is an increase in% scavenging activity as the conc. of the plant extract increase(0.01, 0.05 g %). The TEE and EtOAc were the most potent extract at the two selected dose levels as follows:(46.21%, 69.69%), (47.72%, 68,18%), (36.36%,48.48%), (15.15%,44.69%)and(9.09%, 18.18%) for TEE, EtOAc, $CHCl_3$, methanol & pet.ether respectively(Table 2).

Table 1.Antioxidant activity of TEE of *saracca indica* L. leaves using three different methods

DPPH	154.7µmolTrolox / g dry matter.
FRAP	141.29 µmolTrolox / g dry matter.
ABTS	148.32 µmolTrolox / g dry matter.

TE=Trolox equivalent ; GAE=Gallic acid equi.; Qu E= Quercetin equi.

Table 2. % DPPH antioxidant activity of different extracts of *sarraca indica* L. leaves at two dose levels

Extracts	**% scavenging activity**	
	Concentration	
	0.01g	**0.05g**
TEE	46.21	69.69
Pet. ether	9.09	18.18
$CHCl_3$	36.36	48.48
EtOAc	47.72	68.18
MeOH	15.15	44.69

Screening of the cytotoxic activities of different extracts of *Saraca indica* L. leaves revealed that TEE had activity against MCF-7, HEPG-2&HCT-116 ($IC_{50} \leq 30$ μg/mL [20] and inactive against HeLa cell line; the EtOAc extract had potent cytotoxic activity against HCT-116, and active against MCF-7&HeLa and HEPG-2where the IC_{50} were found to be 0.038, 3.29, 10.14 &19.21ug/ml respectively, compared with standard doxorubicin0.175, 0.155, 0.9& 0.69 ug/ml respectively.

The pet. ether extract had cytotoxic activity against HeLa and MCF-7and inactive against HCT-116 &HEPG-2 with IC_{50} of 7.6, 23.15, 51.41&74.65 ug/ml respectively. The $CHCl_3$ extract has cytotoxic activity against MCF-7, HeLa and HEPG-2 and inactive against HCT-116 the IC_{50} were found to be 2.29 10.37, 28.71&62.54 ug/ml respectively. The successive methanol extract has activity against MCF-7and inactive against HCT-116 with $IC_{50}$of 1.33 & 72.84 respectively. It showed no activity to other cell lines (Table 3)&Fig. 1(A, B,C&D).

Table 3 .Results of the IC_{50} of cytotoxic activity of different extracts of *sarraca indica* L. leaves compared with Doxrubacin as a reference drug

Type of cell line	IC_{50}µg/ml					
	Plant extract					St.
	TEE	Pet. ether	$CHCl_3$	EtOAc	MeOH	Dox.
MCF-7	24.49	23.15	2.29	3.29	1.33	0.155
HEPG-2	23.29	74.65	28.71	19.21	162.76	0.69
HCT-116	69.31	51.41	62.54	0.038	72.84	0.175
Hela	283.69	7.6	10.37	10.14	174.44	0.9

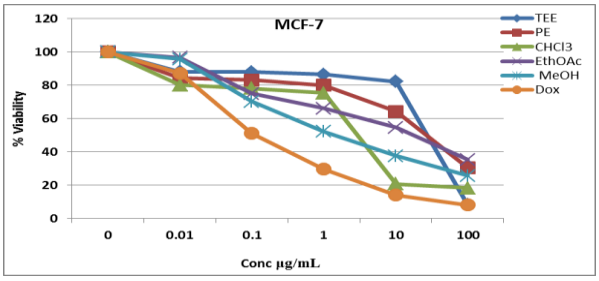


Fig A. MCF-7

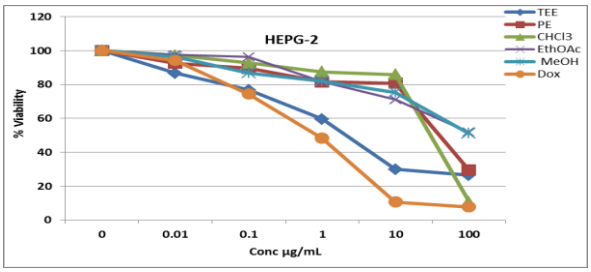


Fig B. HEPG-2

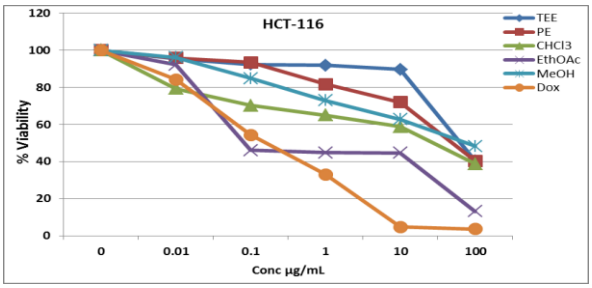


Fig C. HCT-116

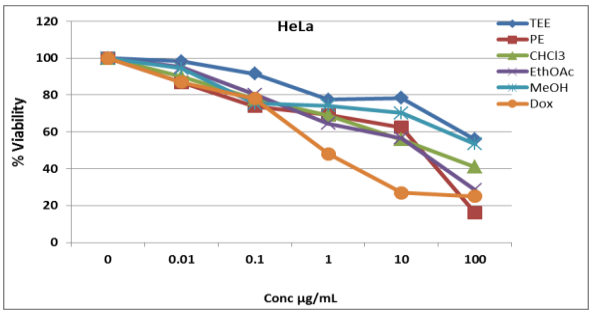


Fig D. HeLa

Fig 1. Results of cytotoxic activities of different extracts of *saraca indica* L. leaves against different cancer cell lines

(Fig A. MCF-7, Fig B. HEPG-2, Fig C.HCT-116 and Fig D. HeLa**)**

Phytochemical screening of the *saraca indica L.* leaves revealed that pet ether & $CHCl_3$ extracts (non polar fractions) were rich in sterols and/or triterpenes while carbohydrates, flavonoides, and tannins were detected in extracts prepared with EtOAc & methanol (polar fractions).The total phenolic and total flavonoid contents in the TEE were found to be 136.26 ± 0.24 mg eq. GA/ g extract, 81.63 ± 0.280mg Qu/g extract respectively. GC/MS analysis of the USF of non- polar extracts (pet ether & $CHCl_3$) of *Saraca indica* leaves (Table 4) resulted in the identification of 34 compounds constituting 86% of the total fraction. The oxygenated compounds constituted(54.43%).The non-oxygenated compounds constituted(31.57%).The major compounds were phytol, dodecane, BHT, α amyrin, β-sitosterol, β–amyrin,&squalene(17.68%,15.37%,9.63%, 8.49%,7.12%,5.06% & 4.14%) respectively. GC/MS analysis of the FAMEF of the plant(Table 5) resulted in the identification of 26 compounds constituting (89.63 %) of the total fraction with the major constituents methyl palmitate (34.59%) and methyl-9, 12,15- octadecatrienoate (33.07%).

Table 4. Results of GC/MS analysis of the USF of *Saraca indica* L. Leaves

No.	Compounds	R_t	RR_t	B.P.	M^+	Area %	M.F.
1	n-Decane	6.55	0.27	57	142	0.35	$C_{10}H_{22}$
2	Trans decahydro naphthalene	7.74	0.32	138	138	0.19	$C_{10}H_{18}$
3	n-Undecene	8.31	0.34	55	154	0.34	$C_{11}H_{22}$
4	n-Undecane	8.64	0.36	57	156	3.92	$C_{11}H_{24}$
5	2- Methyldecahydronaphthalene	8.88	0.37	152	152	0.75	$C_{11}H_{20}$
6	Pentyl cyclohexane	9.33	0.39	83	154	0.46	$C_{11}H_{21}$
7	1-Dodecene	10.33	0.43	97	168	0.06	$C_{12}H_{24}$
8	n-Dodecane	10.65	0.44	57	170	15.37	$C_{12}H_{26}$
9	Hexyl cyclohexane	11.31	0.47	83	168	0.67	$C_{12}H_{24}$
10	n-Tridecane	11.53	0.48	57	184	0.70	$C_{13}H_{28}$
11	1-Tetradecene	13.90	0.58	55	196	0.19	$C_{14}H_{28}$
12	Butylatedhydroxyanisole	15.26	0.64	165	180	0.14	$C_{11}H_{16}O_2$
13	Butylatedhydroxy toluene	15.93	0.67	205	220	9.63	$C_{15}H_{24}O$
14	1-Hexadecene	16.97	0.71	55	224	0.34	$C_{16}H_{32}$
15	1-Octadecene	19.72	0.82	55	252	0.44	$C_{18}H_{36}$
16	6,10,14 -Trimethyl 2-pentadecanone	20.44	0.82	58	268	1.62	$C_{18}H_{36}O$
17	1- Eicosene	22.22	0.93	55	280	0.37	$C_{20}H_{40}$
18	Phytol	23.76	1.00	71	296	17.68	$C_{20}H_{40}O$
19	1-Docosene	24.50	1.03	97	308	0.07	$C_{22}H_{44}$
20	n-Heptacosane	29.48	1.24	57	380	0.15	$C_{27}H_{56}$
21	Squalene	30.72	1.29	69	410	4.14	$C_{30}H_{50}$
22	n-Nonacosane	31.25	1.31	57	408	1.98	$C_{29}H_{60}$
23	n-Triacontane	32.04	1.34	57	422	0.04	$C_{30}H_{62}$
24	ɤ-Tocopherol	32.66	1.37	151	416	0.28	$C_{28}H_{48}O_2$
25	n-Hentriacontane	32.85	1.38	57	436	1.04	$C_{31}H_{64}$
26	Vitamin E	33.30	1.40	165	430	0.12	$C_{29}H_{50}O_2$
27	3β)-Ergost-5-en-3-ol	34.24	1.44	400	400	0.60	$C_{28}H_{48}O$
28	Stigmasterol	34.55	1.45	412	412	0.72	$C_{29}H_{48}O$
29	β-sitosterol	35.25	1.48	414	414	7.12	$C_{29}H_{50}O$
30	Taraxenol	35.45	1.50	218	426	1.13	$C_{30}H_{50}O$
31	β -Amyrin	35.65	1.50	218	426	5.06	$C_{30}H_{50}O$
32	α –Amyrin	36.78	1.54	218	426	8.49	$C_{30}H_{50}O$
33	Fern-7-en-3-beta-ol	37.09	1.56	205	426	0.38	$C_{30}H_{50}O$
34	Epifriedelinol	37.59	1.58	165	428	1.46	$C_{30}H_{52}O$
Total identified constituents						86%	
Hydrocarbons						28.82%	
Sterols						8.44%	
Terpenes						38.74%	
Aromatic						9.77%	
Miscellaneous						0.23%	

Table 5. Results of GC/MS analysis of the fatty acids methyl ester fraction(SF) *Saraca indica* L.Leaves

No.	Compounds	R_t	RR_t	B.P.	M^+	Area %	M.F.
1	Methyl dodecanoate(methyl laurate)	16.20	0.74	74	214	0.98	$C_{13}H_{26}O_2$
2	1,9-Dimethyl nonanedioate (Methyl azelate)	16.42	0.75	152	216	0.29	$C_{11}H_{20}O_4$
3	Methyl tridecanoate	17.45	0.80	74	228	0.32	$C_{14}H_{28}O_2$
4	Dimethyl decanedioate(Dimethyl sebacate)	17.87	0.82	55	224	0.11	$C_{12}H_{22}O_4$
5	Methyl tetradecanoate	18.93	0.87	87	242	6.70	$C_{15}H_{30}O_2$
6	Methyl- 9-pentadecenoate	19.84	0.91	41	254	0.17	$C_{16}H_{30}O_2$
7	Methyl penatdecanoate	20.18	0.92	74	256	0.91	$C_{16}H_{32}O_2$
8	Methyl-9- hexadecenoate(Methyl palmitoleate)	21.19	0.97	268	55	0.26	$C_{17}H_{32}O_2$
9	Methyl hexadecanoate (Methyl palmitate)	21.71	1.00	74	270	34.59	$C_{17}H_{34}O_2$
10	Methyl cis-10-heptadecenoate	22.40	1.03	55	282	0.66	$C_{18}H_{34}O_2$
11	Methyl heptadecanoate(Methyl margarat)	22.68	1.04	74	284	1.60	$C_{18}H_{36}O_2$
12	2- Hydroxyhexadecanoate	22.95	1.05	55	286	0.20	$C_{17}H_{34}O_3$
13	Methyl-9, 12,15-octadecatrienoate	23.79	1.09	79	292	33.07	$C_{19}H_{32}O_2$
14	Methyl octadecanoate (Methyl stearate)	23.96	1.10	74	298	5.93	$C_{19}H_{38}O_2$
15	Methyl nonadecanoate	24.92	1.14	74	312	0.24	$C_{20}H_{40}O_2$
16	Methyl eicosenoate	25.70	1.18	55	324	0.09	$C_{21}H_{40}O_2$
17	Methyl eicosanoate(Methyl arachate)	25.99	1.19	74	326	1.36	$C_{21}H_{42}O_2$
18	Methyl heneicosanoate	26.98	1.24	74	340	0.15	$C_{22}H_{44}O_2$
19	Methyl docosanoate(Methyl behenate)	27.97	1.28	74	354	1.00	$C_{23}H_{46}O_2$
20	Methyltricosanoate	28.89	1.33	74	368	0.17	$C_{24}H_{48}O_2$
21	Methyl tetracosanoate	29.80	1.37	74	382	0.45	$C_{25}H_{50}O_2$
22	Methyl pentacosanoate	30.68	1.41	74	396	0.16	$C_{26}H_{52}O_2$
23	Methyl hexacosanoate	31.53	1.45	74	410	0.17	$C_{27}H_{54}O_2$
24	Methyl octacosanoate	33.15	1.52	74	438	0.08	$C_{29}H_{58}O_2$
25	Methyl nonacosanoate	34.00	1.56	74	452	0.06	$C_{30}H_{60}O_2$
26	Methyl triacontanoate	34.99	1.61	74	466	0.28	$C_{24}H_{48}O_2$
	Total identified constituents					89.63	
	Saturated fatty acid					55.75	
	Unsaturated fatty acid					33.88	

R_t: Retention time, M^+: Molecular weight B.P.: Base peak, RR_t= Retention time relative to Methyl palmitate (R_t=21.71 min)
M.F.: Molecular formula

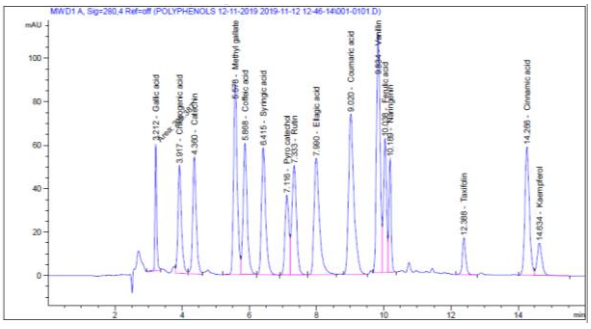


Fig. 2: HPLC chromatogram of phenolic acid and flavonoid contents of standard

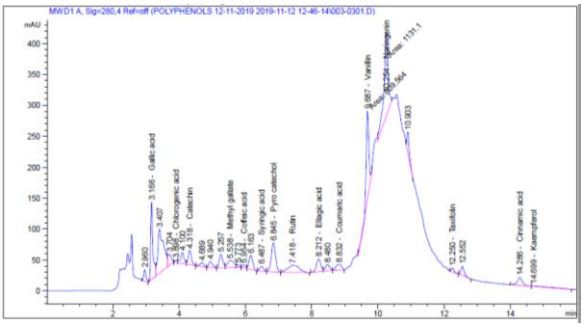


Fig 3 : HPLC chromatogram of phenolic acid and flavonoid contents of EtOAc extract of *saraca indica* L. leaves

HPLC analysis of EtOAc extract of *Saraca indica* L. leaves revealed the identification of 15 compounds with naringenin followed by gallic acid, pyrocatechol, rutin and catechin were the major compounds(18.231%, 9.199%, 6.919%, 4.527% & 2.515%) respectively(Table 6)(Fig. 2&3).

Chromatographic investigation of the EtOAc extract of the plant led to isolation and identification of six compounds; gallic acid(1), methyl gallate (2), rutin (3)querecetin 3-*O*-rhamnoside (4) querectin(5)& kaempferol (6); these compounds were isolated for the first time from the leaves of *saraca indica* L.(Fig 7).The identification of compounds was determined according to R_f values, colour reactions, acid hydrolysis, UVspectrophotometry & EI/MS [17,18]. ^{1}H- and and Co-PC with reference samples, then comparison of their spectroscopic data with previously reported values[17,18, 19] & [21].

Table 6. Results of HPLC analysis of EtOAc extract of *saraca indica* L. leaves

No	R_t	Compounds	Area%	Conc. (µg/ml =µg/18.4 mg)	Conc. (µg/g)
1	3.166	Gallic acid*	9.1988	45.79	2488.68
2	3.898	Chlorogenic acid	0.2813	1.36	73.79
3	4.318	Catechin	2.5147	26.57	1444.28
4	5.538	Methyl gallate*	2.4053	2.25	122.17
5	5.954	Caffeic acid	0.4751	1.07	57.93
6	6.487	Syringic acid	1.0703	2.26	122.93
7	6.845	Pyro catechol	6.9187	41.56	2258.83
8	7.418	Rutin*	4.5273	35.65	1937.50
9	8.212	Ellagic acid	3.0540	10.87	591.00
10	8.832	Coumaric acid	1.7064	1.71	92.88
11	9.687	Vanillin	13.0484	12.64	687.21
12	10.037	Ferulic acid	00	0.00	0.00
13	10.254	Naringenin	18.2308	69.19	3760.47
14	12.250	Taxifolin	0.6349	4.49	244.04
15	14.286	Cinnamic acid	2.2237	1.46	79.17
16	14.699	Kaempferol*	0.6516	3.29	178.77

*Compounds isolated and identified from EtOAc extract

Gallic acid

Blue fluoresce spot on PC under UV light turned to dark blue when sprayed with e$FeCl_3$ Solution, EI-MS Molecular ion peak$[M–H]^-$ at m/z 169, the EI-MS fragmentation pattern: 169.93,79.03,125.00,112.95, 96.01; UV at λ max nm (MeOH): 270.

Methyl gallate

EI-MS ; Molecular ion peak$[M–H]^-$ at m/z 183, the EI-MS fragmentation pattern 183.95,169.96, 152.95, 125.00, 79.00, 51.00 ; UV at λ max (MeOH): 220, 275 nm.

Quercetin-3-O-α-rhamnosyl(1```→6``) β-glucoside (Rutin)

Deep purple spot on PC under UV light ,turning fluorescent yellow when fumed with NH_3 vapour or spraying with $AlCl_3$, with R_f value of 0.380, 0.66 in BAW,15%HOAc respectively. It yield quercetin as an aglycone , rhamnose and glucose as the sugar moieties(Co- PC with authentic samples)on complet acid hydrolysis . UV at λ max nm MeOH: 259, 300 , 356 ; NaOMe: 271 , 326 s h , 411 ; $AlCl_3$: 275 , 305 s h, 422 ;$AlCl_3$/HCl: 269 , 301 s h , 358 , 400 ; NaOAc: 272 , 324 s h , 380 ; NaOAc/H_3BO_3: 262 , 379 . 1H-NMR (500 MHz, DMSO-d6): δ ppm 7.54 (2H, m H-2`/6`), 6.84 (1H, d, J = 9 Hz, H-5`), 6.40 (1H, d, J=1.52Hz, H-8), 6.20 (1H,J=1.52Hz, H-6), 5.35 (1H, d, J=7.04 Hz, H-1``), 4.39 (1H, s, H-1```), 3.90-3.20 (m, remaining sugar protons), 0.99 (3H, d, J=6 Hz, H-6```)

Quercetin-3-O-rhamnoside

Purple spot on PC under UV light ,turning fluorescent yellow when fumed with NH_3 vapour or spraying with $AlCl_3$, with R_f value of 0.69, 0.62 in BAW,15%HOAc respectively. Complet acid hydrolysis yield quercetin as an aglycone and rhamnose as sugar moiety (Co- PC with authentic samples). UV at λ max nm MeOH: 258, 265sh, 350; Na OMe: 272, 322sh, 395; $AlCl_3$: 275, 300sh, 430; $AlCl_3$/HCl : 270, 300sh, 354, 400; NaOAC: 270, 320sh, 370; NaOAC/H_3BO_3: 260, 368.^{1}H-NMR aglycone moiety:δ (ppm) 7.5(2H, m, H-2′ , H-6′), 6.82 (1H, d, *J* = 8.5 Hz, H-5′), 6.36 (1H, d, *J* = 1.5 Hz, H-8), 6.16(1H, d, *J* = 1.5 Hz, H-6). Sugar moiety: δ(ppm) 5.41 (1H, s, H-1″), 3.5-3.2 (m, sugar protons), 0.99 (3H, d, *J* = 6 Hz, rhamnosyl CH_3).

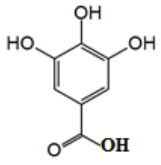
Quercetin

Yellow spot on PC intensified on exposure to NH_3 vapour or spraying with $AlCl_3$ with R_f value of 0.66, 0.08 Rf value of UV at λ max nm MeOH: 256, 265sh, 354; Na OMe: 273, 322sh, 394;

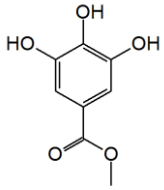
$AlCl_3$: 274, 300sh, 432; $AlCl_3$/HCl : 271, 300sh, 353, 400; NaOAC: 270, 320sh, 371; NaOAC/ H_3BO_3: 262, 369.

kaempferol

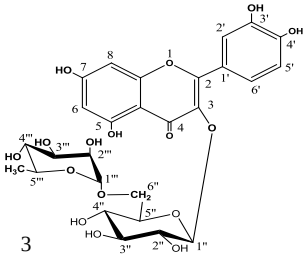
yellowish green spot intensified on exposure to NH3 vapour or spraying with AlCl3, with Rf value of 0.74 , 0.11; UV at λ max nm :MeOH: 266, 297 sh, 363; NaOMe:284,330,420; AlCl3 265, 275, 308, 322, 350,419;AlCl3/HCl :265, 275, 304, 350, 419; NaOAC : 275,283,336; NaOAC /H_3BO_3 :266,298,347,373



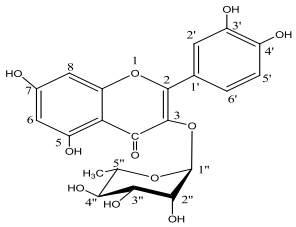
1



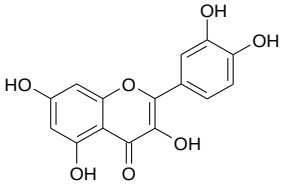
2



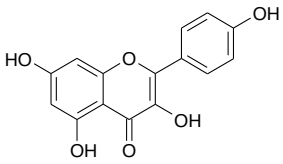
3



4



5



6

Discussion

Cancer is one of the leading causes of death; it is characterized by irregular cell proliferation. According to the World Health Organization [22] more than 14 million people diagnosed with cancer and 8 million died in 2012. High mortality and incidence make it important public health and economic issue which requires effective prevention. The conventional therapy for cancer (radiotherapy and chemotherapy) have proved many side effects on all body organs, this leads us towards the discovery of another way of treatments to overcome these side effects. The use of medicinal plants as an alternative way of treatment has various advantages as plant-derived compounds are more tolerant and non-toxic to the normal human cells. Many studies [23] were done concerning the effect of naturally occurring compounds that proved cytotoxic activity or have the potential to destroy cancer cells. So medicinal plants are in high demand and several species of medicinal plants have been investigated and selected for the preparation of cancer medicines [24]. Oxidants and free radicals are inevitably produced during the physiological and metabolic processes in the human body; the later has defensive antioxidant mechanisms that vary according to cell and tissues. These mechanisms include; natural enzymes SOD, CAT and Glutathione peroxidase (GPx), as well as antioxidants substances such as vitamins, carotenoids, polyphenols, and other natural antioxidants. Among the several classes of phytochemicals, interest has focused on the antioxidant properties of the polyphenols that are found in most plants. Plants, vegetables, and spices used in folk and traditional medicine have gained wide acceptance as one of the main sources of prophylactic and chemo preventive drug discoveries and development [25]

Reactive species (RS) of various types are powerful oxidizing agents, capable of damaging DNA and other biomolecules. The increase in the formation of RS can promote the development of malignancy. Evidence suggests that the plant kingdom is considered a good candidate for chemoprevention and cancer therapy due to the high concentration and wide variety of antioxidants compounds with the capability to inhibit the cell proliferation of different cancer cells *in vitro* such as colon breast, cervix, liver, skin, fibroblasts, and many other malignant cells; studies have indicated that antioxidants can be employed efficiently as chemo preventives and as effective inhibitors of cell proliferation, promoting cell apoptosis, and increasing detoxification of enzymes, and inhibiting gene expression and scavenger of reactive oxygen species (ROS) [26]

Medicinal plants contain wide ranges of secondary metabolites which include flavonoids, flavones and others [24]. These bioactive compounds are mainly responsible for the antioxidant prosperity of medicinal plants. The increasing side effects and expensive medications have titled the focus of researches on herbal medicines. Therefore, in our research, we investigate the plant *Saraca indica* leaves L for its activity as antioxidant& cytotoxic which were reported as some of the Indian folk medicine uses..

From our previous results the TEE exhibited cytotoxic activity to MCF-7&HEPG-2 and inactive against HCT-116& HeLa; while fractionation of the TEE by organic solvents to obtain pet.ether, $CHCl_3$, EtOAc& methanol fractions led to appearance of potent cytotoxic activities in some fraction this is explained on the bases of TEE contain many active constituents that

may act as antagonist to each other while fractionation of TEE to different fractions led to separating the antagonistic effect of the different constituents from each other and consequently the activity of some of the successive extracts increased. From the previous results of cytotoxicity we can conclude that; Since plant extracts are considered to be active as anti-cancer agents when $IC_{50} \leq 30$ μg/mL [20], thus the EtOAc extract considered to be the most active anti-proliferative agent that should be recommended for further clinical studies. These results are consistent with the reported Indian folk medicine use of the plant as anticancer [3, 4, 5].

Phytochemical screening of the plants revealed the presence of carbohydrates, flavonoids, and sterol/or triterpenes. Natural polyphenolic compounds have attractive consideration for their cancer-preventive effect [27] The isolated flavonoids and or phenolic acids(gallic acid, methyl galate) from the plant leaves, in addition to the different identified constituents in the non - polar fraction were all reported for their antioxidant and cytotoxic activity

Rutin is a flavonoid which is present in many natural plants, it was reported to cause cell cycle arrest and induce apoptosis in many types of human cancer cell lines, it protect human vein endothelium cells against H_2O_2- induced apoptotic cell death [28].

Quercetin is a flavonoid which is widespread in numerous plants, it is utilized in many different cultures for its nervous systemand anticancer effects. The oxidative kinase and cell cycle inhibitory, apoptosis-inducing effects of quercetin are essential for its anticancer effects [29].

The unsaponifiable fraction recorded higher percentage of phytol, dodecane, BHT, α amyrin, β-sitosterol,β –amyrin, Squalene, & epifriedelinol (17,68%, 15.37, 9.63%, 8.49%, 7.12%,5.06%, 4.14% & 1.46 respectively.

Butylated hydroxytoluene (BHT) was reported for its antioxidant activity [30] and has been found to inhibit photocarcinogenesis in animal models [31].

Phytol is diterpene alcohol it has been reported for many pharmacological activities including cytotoxic, antitumorous, antimutagenic and anti-teratogenic [32].

Phytol is screened against several cell lines it was found that it is a concentration-dependent response in all cell lines, demonstrating to be most effective against MCF-7 with IC_{50} 8.79 ± 0.41 μM; the IC_{50} values towards other tumors cell line ranged from 15.51 to 69.67 μM. It also exerted weak activity against PC-3 cells, (IC_{50} 77.85 ± 1.93 μM) [33] [34].

Squalene is the precursor of cholesterol biosynthesis, it has unique physical property and a wide variety of physiological functions such as anticancer, anti-hypercholesterolemia and antioxidant potential [35] .

Phytosterols were reported for their inhibitory effect on lung, stomach, as well as ovarian and breast cancer. Phytosterols seem to act through inhibition of carcinogen production, cancer-cell growth, angiogenesis, invasion and metastasis, and through the promotion of apoptosis of cancerous cells. Phytosterol consumption may also increase the activity of antioxidant enzymes and thereby reduce oxidative stress. In addition to altering cell-membrane structure and function [36].

Sitosterol is a valuable component of the human diet that possesses anticancer or cancer-preventive properties/effects due to its interaction with various cellular targets and pathways. There were reported data about the *in vitro* anticancer activity against HT-29 cells, where β-sitosterol demonstrated 2.6 times higher selectivity than methotrexate [37].

Epifriedelinol was reported for its anticancer activity against cervical cancer through the induction of apoptosis [38].

9,12,15- Octadecatrienoic acid is a polyenoic fatty acid and it was reported for many pharmacologic activities including cancer-preventive [39] This study is considered to be the first report deals with the phytochemical constituents of *Saraca indica* L. leaves as well astheir cytotoxic and antioxidant activities.

Conclusion

Screening of the antioxidant activity of the different extracts of *saracca indica* L. leaves indicates that all fractions have variable antioxidant activities with TEE and EtOAc were the most active; additionally, the TEE had cytotoxic activity against MCF-7, HEPG-2& HCT-116 ($IC_{50} \leq 30$ μg/mL[40] and inactive against HeLa cell line; the EtOAc extract had potent cytotoxic activity against HCT-116, and active against other cell lines. The study of the constituents of the non- polar extract through GC/MS analysis led to the identification of 34 compounds in USF and 26 compounds in the SF. HPLC analysis of the EtOAc extract showed the presence of fifteen and in addition to isolation of seven phenolic compounds from the same extract for the first time. Based on the data of the current study the previous two extracts could be considered as natural antioxidant cytotoxic agents.

Acknowledgment

The authors extend special appreciation to the National Research Centre, Dokki, Giza, Egypt for offering the facilities and funds No. 2/5/7 for this study. This work was extracted from the Ph.D.

Thesis of Wedian El-Sayed Mohamed Ashour, Pharmacognosy Department. 2015, National Research Centre

References

1. Hooker, J.D., *Flora of British India, Vol. II*. Flora of British India, Vol. II., 1879.
2. Pradhan, P., et al., *Saraca asoca (Ashoka): a review*. Journal of chemical and pharmaceutical research, 2009. **1**(1): p. 62-71.
3. Srivastava, G., G. Bagchi, and A. Srivastava, *Pharmacognosy of Ashoka stem bark and its adulterants*. International Journal of Crude Drug Research, 1988. **26**(2): p. 65-72.
4. SABUJ, M.Z.R., *the degree of Master of Science (MS) in Zoology (Crop Protection and Toxicology), University of Rajshahi*. 2016, University of Rajshahi-6205 Bangladesh.
5. Bhandary, M., K. Chandrashekar, and K. Kaveriappa, *Medical ethnobotany of the siddis of Uttara Kannada district, Karnataka, India*. Journal of ethnopharmacology, 1995. **47**(3): p. 149-158.
6. Nadkarni, K.M., *[Indian materia medica]; Dr. KM Nadkarni's Indian materia medica: with Ayurvedic, Unani-Tibbi, Siddha, allopathic, homeopathic, naturopathic & home remedies, appendices & indexes. 1*. Vol. 1. 1996: Popular Prakashan.
7. Mishra, A., et al., *Phytochemical and pharmacological importance of Saraca indica*. Int. J. Pharm. Chem. Sci, 2013. **2**(2): p. 1009-1013.
8. Kumar, S., et al., *Evaluation of antihyperglycemic and antioxidant activities of Saraca asoca (Roxb.) De Wild leaves in streptozotocin induced diabetic mice*. Asian Pacific Journal of Tropical Disease, 2012. **2**(3): p. 170-176.
9. Yamaguchi, T., et al., *HPLC method for evaluation of the free radical-scavenging activity of foods by using 1, 1-diphenyl-2-picrylhydrazyl*. Bioscience, biotechnology, and biochemistry, 1998. **62**(6): p. 1201-1204.
10. Benzie, I.F. and J. Strain, *[2] Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration*, in *Methods in enzymology*. 1999, Elsevier. p. 15-27.
11. Re, R., et al., *Antioxidant activity applying an improved ABTS radical cation decolorization assay*. Free radical biology and medicine, 1999. **26**(9-10): p. 1231-1237.
12. Skehan, P., et al., *New colorimetric cytotoxicity assay for anticancer-drug screening*. JNCI: Journal of the National Cancer Institute, 1990. **82**(13): p. 1107-1112.
13. Marinova, D., F. Ribarova, and M. Atanassova, *Total phenolics and total flavonoids in Bulgarian fruits and vegetables*. Journal of the university of chemical technology and metallurgy, 2005. **40**(3): p. 255-260.
14. Sembiring, E.N., B. Elya, and R. Sauriasari, *Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of Caesalpinia bonduc (L.) Roxb*. Pharmacognosy journal, 2018. **10**(1).
15. Abou Zeid, A.H., et al., *Bioactivity and chemical constituents of Gleditsia triacanthos L. leaves*. Egyptian Pharmaceutical Journal, 2018. **17**(2): p. 70.
16. Adams, R.P., *Identification of essential oil components by gas chromatography/mass spectrometry*. Vol. 456. 2007: Allured publishing corporation Carol Stream, IL.
17. Mabry, T., K.R. Markham, and M.B. Thomas, *The systematic identification of flavonoids*. 2012: Springer Science & Business Media.
18. Markham, K.R., *Techniques of flavonoid identification*. Vol. 36. 1982: Academic press London.
19. AGRAWAL, P.K., *Carbon-13 NMR of flavonoids*. Studies in Organic Chemistry, 1989. **39**: p. XVI-564.
20. Suffness, M., *Assays related to cancer drug discovery*. Methods in plant biochemistry: assays for bioactivity, 1990. **6**: p. 71-133.
21. Markham, K., ^ *1H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulfoxide*. The flavonoids: advances in research, since 1986, 1994.
22. Sisay, M., et al., *Evaluation of rational drug use based on World Health Organization core drug use indicators in selected public hospitals of eastern Ethiopia: a cross sectional study*. BMC health services research, 2017. **17**(1): p. 1-9.
23. Greenwell, M. and P. Rahman, *Medicinal plants: their use in anticancer treatment*. International journal of pharmaceutical sciences and research, 2015. **6**(10): p. 4103.
24. Sumner, J., *The natural history of medicinal plants*. 2000: Timber press.
25. Valadez-Vega, C., et al., *The role of natural antioxidants in cancer disease*, in *Oxidative Stress and Chronic Degenerative Diseases-A Role for Antioxidants*. 2013, IntechOpen.

26. Halliwell, B., *Free radicals in biochemistry and medicine.* Reviews in Cell Biology and Molecular Medicine, 2006.
27. De Santis, G., et al., *Evaluation of two groups of quinoa (Chenopodium quinoa Willd.) accessions with different seed colours for adaptation to the Mediterranean environment.* Crop and Pasture Science, 2019. **69**(12): p. 1264-1275.
28. Lin, J.P., et al., *Rutin inhibits human leukemia tumor growth in a murine xenograft model in vivo.* Environmental toxicology, 2012. **27**(8): p. 480-484.
29. Dajas, F., *Life or death: neuroprotective and anticancer effects of quercetin.* Journal of ethnopharmacology, 2012. **143**(2): p. 383-396.
30. Takahashi, O. and K. Hiraga, *Effect of butylated hydroxytoluene on the lipid composition of rat liver.* Toxicology, 1981. **22**(2): p. 161-170.
31. BLACK, H.S. and M.M. MATHEWS-ROTH, *Protective role of butylated hydroxytoluene and certain carotenoids in photocarcinogenesis.* Photochemistry and photobiology, 1991. **53**(5): p. 707-716.
32. Islam, M.T., et al., *Phytol in a pharma-medico-stance.* Chemico-Biological Interactions, 2015. **240**: p. 60-73.
33. Pejin, B., V. Kojic, and G. Bogdanovic, *An insight into the cytotoxic activity of phytol at in vitro conditions.* Natural product research, 2014. **28**(22): p. 2053-2056.
34. Kim, C.W., et al., *Activation of caspase-9/3 and inhibition of epithelial mesenchymal transition are critically involved in antitumor effect of phytol in hepatocellular carcinoma cells.* Phytotherapy Research, 2015. **29**(7): p. 1026-1031.
35. Narayan Bhilwade, H., et al., *Squalene as novel food factor.* Current Pharmaceutical Biotechnology, 2010. **11**(8): p. 875-880.
36. Woyengo, T., V. Ramprasath, and P. Jones, *Anticancer effects of phytosterols.* European journal of clinical nutrition, 2009. **63**(7): p. 813-820.
37. Xu, J., et al., *TRIM29 promotes progression of thyroid carcinoma via activating P13K/AKT signaling pathway.* Oncology reports, 2017. **37**(3): p. 1555-1564.
38. Yang, J., J. Fa, and B. Li, *Apoptosis induction of epifriedelinol on human cervical cancer cell line.* African Journal of Traditional, Complementary and Alternative Medicines, 2017. **14**(4): p. 80-86.
39. Vohra, A. and H. Kaur, *Chemical investigation of medicinal plant Ajuga bracteosa.* J Nat Prod Plant Resour, 2011. **1**(1): p. 37-45.