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LC-MS/MS analyses of *Khaya grandifoliola* and *in vitro* Antioxidant Activity and Cytotoxicity of *Khaya senegalensis* and *Khaya grandifoliola* against Ehrlich Ascites Carcinoma Cells

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Abstract

Synthetic drugs of many chronic diseasesare often accompanied by harmful side effects. Therefore, this study aimed to evaluate the potential *in vitro* antioxidant and antitumor activities of *K. senegalensis* and *grandifoliola* (Meliaceae), and their preliminary phytochemical screening. Also, a fast precise LC-MS/MS screening was performed to identify the constituents of the second species. It is worth mentioning that the phytochemical and biological examinations were carried out on the total 70% methanol extracts of the aerial parts and their MeOH-soluble portions (MSP). LC-MS^2 of *K.grandifoliola*-MSP led to identification of 33 flavonoids, including 10 aglycones (**1**–**10**) and 23 glycosides (**11**–**33**), mostly of 3-*O*-kaempferol or quercetin, 2 chalcones (**30**,**31**) and 1 anthocyanin (**33**). Eleven phenolic acid derivatives (**34**–**44**) and 1 stilbene glycoside (**92**) were identified as polyphenolics-type alongside 3 coumarins (**45**–**47**). Other major classes identified as 13 organic acid (**48**–**60**) 17 purine and pyrimidine (**61**–**77**), 6 amino acid (**78**–**83**), and 8 sugars and polyhydric alcohols (**84**–**91**) derivatives. The identification depends on the matching of R_t-values, molecular and fragment/s monoisotopic masses and fragmentation pathways with the literature and library databases. Antioxidant activity was determined using several protocols and antitumor activity evaluated against Ehrlich ascites carcinoma cells (EACC). By all antioxidant assays, the extracts revealed significant activity, relative to the reference agents that can be attributed to their high polyphenols contents. The total *Khaya* species extracts recorded higher activity than the corresponding MSP. The promising antioxidant and antitumor findings of all extracts revealed good broad-spectrum activities due to the relatively low concentrations examined relative to the standard drugs. Therefore, current extensive isolation and identification studies are being performed for major polyphenols alongside deep investigation of their antioxidant and anticancer potentials. Results provide evidence that validates the use of *K. senegalensis* in pharmaceutical and nutrition industries.

Keywords: Meliaceae, *Khaya*, Polyphenolics, Phytochemical profiling, LC-MS/MS, Antioxidant, Ehrlich ascites carcinoma

1. Introduction

The family Meliaceae is a flowering plant family of mostly trees and shrubs in the order Sapindales, comprising about 50 genera and 1400 species. It is widely distributed subtropical and tropical angiosperm family occurring in a various habitats, from rain forests and mangrove swamps to semi deserts [1,2]. *Khaya* species are native to Madagascar and tropical Africa and have been introduced to Southern Asia and Australia from central Africa [3]. The genus *Khaya* consists of 6 recognized species: *K. anthotheca* (Welw.) C.DC., *K. grandifoliola* C.DC., *K. ivorensis* A. Chev., *K. senegalensis* (Desr.) A. Juss, *K. madagascariensis* Jum. & H. Perrier and *K. nyasica* Staph ex Baker F [3,4], that are of high commercial and economic importance. It is confirmed that *K. grandifoliola* stem bark is used by traditional healers in Cameroon to cure salmonellosis and malaria. Reactive oxygen species (ROS) cause harm to numerous physiological systems in the human body. Numerous environmental (stress, tobacco, air pollutants, radiation, etc.) and other internal factors, such as mitochondrial respiratory chain, NADPH oxidase, and xanthine oxidase, contribute to the generation of these ROS [5]. It is

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reported that when a human host infected with the Plasmodium parasite is stimulated to produce an excess of free radicals to fight against infection [6]. These free radicals are just as harmful to the host as they are to the parasite. Therefore, having a medication with natural antioxidant and antiplasmodial qualities will be crucial. Also, cancer is a dreadful disease and any practical solution in combating this disease is of paramount importance to public health[7]. It is currently the leading cause of death globally [8] and the number of deaths from cancer is on the rise daily [9]. Cancer is a disease that is characterized by proliferation of the body cells, due to failures in cellular modulation and obstruction of cell cycle progression, and thereby eliciting malignant tumor cells formation with the possibility of becoming metastatic [10]. Recently bioactive compounds of medicinal plants with anticancer potentials are attracting researchers' attention in the fight against cancer. Plant extracts are widely used in Nigeria as important sources of chemotherapeutic agents in spide of the use of synthetic drug by vast majority of the populace. Medicinal plants have been in continuous use over the years for the management of cancer [11], in most developing countries of the world including Nigeria. In addition, the alternative use of readily available and inexpensive medicinal plants is the panacea to the toxic side effects associated with synthetic drugs [7,12–14]. More than 80% of the population continues to treat themselves with medicinal plants in Africa [15–17]. This situation leads to the consideration of medicinal plants as an alternative to conventional synthetic drugs, and as a bio safe solution against various diseases [18,19]. Indigenous knowledge on healing attributes of plants has been transmitted from generation to generation, and today, they serve as the basis for plant based drug discovery research [20]. Clinical studies and phytochemical screening have established the antitumor activity of herbal remedies against different types of cancers [7,21,22]. There are over 114,000 plant extracts that are being analysed for their anticancer activity in various cancer institutes. Accordingly, there is a pressing need to carry out conclusive investigations to establish whether these extracts exhibit anticancer activityand applied as chemotherapeutic agents [11,23,24]. *Khaya* species have been used traditionally for treating several ailments, including malaria, rheumatism, fever and back pain in Africa [25]. Phytochemical screening of *Khaya* detected high contents of fatty acids, limonoids, polyphenols (flavonoids, coumarins, lignans, proanthocyanin-dins), triterpenes, chromones, alkaloids, anthra-cenosides, carbohydrates, saponins, sterols and cardiac glycosides [26,27]. However, cytotoxicity was shown by most of the species as the major activity in case of therapeutic actions[28,29]. The extensive traditional use of *Khaya* species has encouraged scientists to explore several biological activities including insecticidal activity [30], antimalarial [31], anti-oxidant [28], antifeedant [32,33]. Because of the large number of different secondary metabolites that have been reported from *K. grandifoliola*, *K. senegalensis* and their broad structural variation, (different types of polyphenols, limonoids, terpenoids and sterols)[3,34–40], this study represents a preliminary phytochemical screening for their methanol-soluble portions (MSP) of the 70% aq. methanol aerial parts extracts and an accurate phytochemical determination by UPLC/qTOF-HRESI-MS/MS for *K.grandifoliola* alongside evaluation of the *in vitro* antioxidant and cytotoxicity for both species.

2. Materials and methods

2.1. Chemicals and reagents

Concerning the *in vitro* studies, chemical reagents and solvents used as HPLC- and/or analytical grades and delivered from Sigma-Aldrich Co. (St. Louis, MO, USA), Thermo-Fisher Scientific (Waltham, MA, USA) or Merck (Darmstadt, Germany). Details of all chemicals and reagents are described before [41].

2.2. Plant material

Aerial parts of *K. senegalensis* were collected from the Ismallia University Garden, Egypt, on 10 June 2020, while *K. grandfoliola* aerial parts collected from the zoo, Giza, Egypt, on 6 July 2021. The plants authenticated by Dr Reem Samir Hamdy, Professor of Taxonomy and flora, Botany Department, Faculty of Science Cairo University, Egypt. The voucher specimens, Reg. No. R-Ks -I, and R-Kg -II of the two plants, respectively, were deposited within the Herbarium, Botany Department, Faculty of Science Cairo University, Egypt. The material was dried in a well aerated shaded place, powdered, and saved separately in tightly closed containers.

2.3. Extraction

Air-dried *K. senegalensis* aerial parts (2 Kg) were ground using a pestle and mortar to a fine powder that was exhaustively extracted with 70% aq. MeOH (7 x 3L, 70◦C) under reflux. The collective extract cuts were filtered and concentrated in vacuo to produce dry total extract residue of 412.8 g. It was taken with hot pure methanol under reflux (20x 0.5 L, 60◦C), giving dry methanol soluble portion (MSP) of 260 g. Concerning to the aerial parts of the second plant (*K. grandfoliola*), an air-dried amount of 250 g was extensively extracted with 70% aq. MeOH in a reflux conditions (4 x 2L, 70◦C). Similarly, the total crude extract was collected, filtered, and

concentrated in vacuo to produce a dry residue of 68.66 g. Thereafter, it was taken with hot pure methanol under reflux (2 x 1 L, 60◦C), giving dry MSP of 41.269 g. Finally, both samples were stored at 4 ◦C until analysis.

2.4. Preliminary phytochemical screening of both Khaya species

Air- dried powdered aerial parts of two *khaya* species in Egypt *K. senegalensis and K. grandfoliola* were separately screened for the presence of volatile substances [42], carbohydrates and/or glycosides [43], alkaloids and /or nitrogenous bases [43], saponins [43], anthraquinones [43], unsaturated sterols and/or triterpenes [43], coumarins [43], tannins [44], flavonoids [43,45,46] and iridoids [43]. As well, phenolic compounds were screened by 2D-PC using upper layer of BAW (n-BuOH-AcOH-H_2O, 4:1:5) for the first run and 15% aqueous AcOH for the second run [47]. Visualization of the spot was carried out by UV light and spraying with different spray reagents (ammonia, $AlCl_3$, $FeCl_3$).

2.5. UPLC/ESI-qTOF-HRMS/MS analysis

The measurements were performed at the proteomics and metabolomics unit (Children's Cancer Hospital (CCHE 57357), Cairo, Egypt). LC-MS system composed of a standard HPLC interface (Exon LC, Sciex) combined with a quadruple time-of-flight (qTOF) mass spectrometer (Triple TOF 5600+, Sciex) equipped with HR-TOF scan capabilities. This system was set up in negative mode such that enabled for MS/MS selective fragmentation analysis and the collection of structural documents [48,49]. The determination and interpretation of MS data were executed by Analyst TF/1.7.1 and MS-DIAL/4.8 open-source software alongside Respect negative (1573 records) reference databases. Enhanced product ion (EPI) scan in a linear qTOF with information dependent data acquisition (IDA) gave capability for association of MS fragment data even from minor metabolites.LC separation was obtained with a Waters HPLC column: X select HSS T3 (2.1x150 mm, 3.5µm) that was reserved at a temperature of 40°C. A Phenomenex in-line filter disks pre-column (3.0 mm x 0.5µm) was used. The mobile phases are designed as: A) 5 mM $HCOONH_4$ buffer (pH=8) in 1% MeOH, B) 100% MeCN. An injection volume (10 µl) of 2.5 µg/µl solution in H_2O-MeOH-CH_3CN (50: 25: 25 v/v) was utilized with a flow rate of 0.3 ml/min, after vortex for 2 min, 10 min ultra-sonication to prepare stock solution sample (50 mg/ml) and then dilution 50 to 1000 µl. The separation based on a linear gradients time stages given in Table 1. HPLC grade solvents: MeOH, HCOOH, and NaOH for pH adjustment were delivered from Fisher Scientific (UK); $HCOONH_4$, and CH_3CN from Sigma-Aldrich (Germany); H_2O Milli-Q (Millipore, USA).

Table 1.Time program of UPLC/HRESI-MS analysis for MSP of *K. grandifoliola*

Time/min	0	1	21	25	25.01	28
%A	90.0	90.0	10.0	10.0	90.0	90.0
%B	10.0	10.0	90.0	90.0	10.0	10.0

MSP= MeOH-soluble portion

2.6. Antioxidant assays

DPPH, ABTS free radical scavenging assays, reducing power assay, and FRAP antioxidant capacity assay were evaluated following the previous procedures [50,51], The used concentration for each assay was identified according to obtaining the highest activity using the lowest concentration.

2.7. Cytotoxicity investigations using EAC cells

The Trypan blue exclusion method was used to evaluate the two *Khaya* species extracts *in vitro* cytotoxicity [50]. To summarize, sterile test tubes containing two millions Ehrlich ascites carcinoma (EAC) cells were aspirated from the intraperitoneal inoculated female Swiss albino mice. The cells were then incubated with varying concentrations of two *Khaya* species extracts (10,25,50 and 100 µg/ml) for 120 min at 37°C in CO_2 incubator. Following the incubation period, Trypan blue dye (0.4% in PBS) was introduced, and a hemocytometer was used to count the total number of dead (stained) and viable (unstained) cells. The standard drug vincristine was used as positive control. The percentage of cytotoxicity was then computed using the following formula:

% Cytotoxicity = 100× (T dead – C dead)/T total

Where T dead is the number of dead cells in the treated samples, C dead is the number of dead cells in the control and T is the total number of dead and live cells in the treated samples.

2.8. Statistical analysis

Standard deviation and Mean were calculated employing an Excel worksheet.

3. Results

3.1. Preliminary phytochemical screening

Both *Khaya* species were screened by conventional phytochemical and chromatographic screening tests for the aerial parts extracts (*K. senegalensis* and *grandifoliola)* according to the literature reported in section 2.4 [42–47]. The results demonstrated the presence of all tested classes in abundant ratios (+++), except for saponins and flavonoids (Table 2). The first class was noticed as trace amounts in both species, while the second one observed as trace amount in the second species only.

Table 2. Preliminary phytochemical screening findings of the MSP for *K. senegalensis and K. grandifoliola*

Class	Test	Results		Observations		Ref.
		K. senegalensis	*K. grandfoliola*	*K. senegalensis*	*K. grandfoliola*	
volatile substances	Micro sublimation	Sphere crystals + + +	Sphere crystals + + +	Contains sublimated compounds	Contains sublimated compounds	[42]
Carbohydrate or/glycoside	Molisch's test	+ + +	+ + +	Violet ring	Violet ring	[43]
	Fehling test	+ + +	++	Dark red color	Red color	
Alkaloids	Mayer's reagent	+ + +	+ + +	Brown	Green	
	Dragendrff reagent	+ + +	+ + +	Orange brownish	Orange brownish	[43]
	Wagner's reagent	+ + +	+ + +	Yellow brownish	Green brownish	
Anthraquinones	Borntragor's	+ + +	+ + +	Red color	Dark red color	[43]
Coumarins	----------	+ +	+ +	Fluorescence yellow	Fluorescence yellow	[43]
Saponins	Froth	+	+	1.8 cm	2 cm	[43]
	Blood haemolysis	+	+	1.8 cm	2 cm	
Flavonoids	Shinoda's	+ + +	+	Dense pink	Magnetic red	[43]
	Free flavonoids	+ + +	+	Dark yellow color	yellow color	[45]
	Combined flavonoids	+ + +	+	yellow color	Faint yellow color	[46]
Unsaturated sterols or/terpenes	Libermannburchared's	++	+ + +	Brown ring	Strong brown ring	[43]
	Salkowisk's	+ +	+ + +	Brown ring	Strong brown ring	
Tannins	$FeCl_3$	+ + +	+ + +	Dark green	Dark green	[44]
	Matchstick	+ + +	+ + +	Red color	Red color	
	Vanillin HCl	+ + +	+ + +	Red and ppt	Red and ppt	
	Gelatin	+ + +	+ + +	white ppt	white ppt	
Iridoids	----------	+ + +	+ +	Red color	Brownish yellow	[43]

(-) Absent, (+) Present in traces, (++) Present, (+++) Present in abundance

3.2. LC-ESI-qTOF-HRMS profile of K. grandi-foliolaaerial parts

UPLC/ESI-qTOF-HRMS/MS tool was optimized to identify different 105 metabolites, belonging to many structural-types metabolites with high accuracy from the MSP of *K. grandifoliola*aerial part crude extract (Table 3). In general, Rt-values, monoisotopic masses of both molecular and some selective fragment ions with their relative abundances constituted the main efficient identification parameters after matching with the available scientific literature and the MS-DIAL 3.70 open-source software [52]. However, respect negative (1573 records) databases were used as the reference database for the identification of the products. The TIC and BPC gave an idea about the enrichment of the extract sample investigated with different metabolites classes (Fig. 1). It is worthy that 45.71% of the total represented peaks were interpreted for phenolic compounds because of their high stability in negative ESI-mode of ionization as phenolate anions. Among the important output LC/MS data, XIC and MS^2 spectra of all major identified 99 metabolites were presented in figures 1S–99S, which exhibited an idea about their relative ratios and the stability of probable corresponding ions. Practically 48 polyphenolics were identified, including 33 flavonoids (10 aglycones **1**–**10**, 23 glycosides **11**–**33**) and 11 phenolic acids (**34**–**44**), 3 coumarins (**45**–**47**), and a stilbene (**92**) (Figs. 1S–47S, 92S). In spide of the identification based mainly on the blind matching of the experimental output data and the stored library database and literature, the selective fragment ions in each metabolite can be followed and explained for all other identified metabolites (Figs. 48S–99S).

3.3. Antioxidant activity

It is recommended to conduct many assays to ascertain the antioxidant mechanism and activity when evaluating chemical antioxidants. This has importance for natural products, as their antioxidant properties might originate from a combination of substances acting via many pathways [53]. Therefore, assays for DPPH radical, reducing power ability, ferric reducing power, and ABTS radical scavenging activity are widely used to detect the antioxidant capacities of natural products and are employed in current investigations.

Table 3. Negative UPLC-ESI-qTOF-HRMS/MS identification of the metabolites in the MSP of *K. grandifoliola*

Peak No.	R_t (min)	$[M-H]^-$ *(m/z)*	Area	Error (ppm)	MF	MS^2 Fragments *(m/z)*	Metabolite
Flavonoids							
A) Aglycones							
1.	1.124	317.0545	455750	0.8	$C_{15}H_{10}O_8$	164.9884:71 249.01341:107 317.04916:1132	Myricetin
2.	5.107	315.1106	1889345	-4.8	$C_{16}H_{12}O_7$	161.04173:71 269.10489:250 315.10583:625	5,7,4′-Trihydroxy-3′-methoxy-flavonol
3.	9.662	301.0332	234721	5.6	$C_{15}H_{10}O_7$	179.00253:107 301.0361:626	Quercetin
4.	14.310	299.0565	190379	-0.7	$C_{16}H_{12}O_6$	165.01982:214 243.0687:107 256.03787:143 271.05722:250 284.03125:143 299.05698:686	3,5,7-Trihydroxy-4′-methoxyflavone
5.	5.453	289.0699	276296	6	$C_{15}H_{14}O_6$	112.98931:107 167.0304:71 180.98212:107 205.04945:107 289.07278:402	(+)-3, 5,7,3',4'-Pentahydroxyflavan
6.	10.335	271.0616	823928	-0.4	$C_{15}H_{12}O_5$	119.0531:214 125.0214:73 151.00255:478 177.01585:214 253.0428:74 271.05875:1793	Naringenin
7.	6.681	269.14	72053	-0.3	$C_{15}H_{10}O_5$	269.1474:71	Apigenin
8.	10.981	285.0385	86691	-0.1	$C_{15}H_{10}O_6$	285.04572:521	Luteolin
9.	4.630	289.0722	4928059	-0.2	$C_{15}H_{14}O_6$	109.02864:322, 112.98474:404, 123.04477:465 125.01697:322, 125.02487:366 135.04588:107, 151.03935:468 161.06171:214, 164.01434:252 179.03613:514, 180.97627:179 187.03323:179, 202.06738:180 203.07639:408 205.04932:658 221.08369:179 227.07446:214 245.08052:1741 247.07426:107 289.06772:3440	Catechin
10.	4.333	577.1329	386574	1.6	$C_{30}H_{26}O_{12}$	125.02808:71 289.08929:54 407.08316:179 425.07129:455 576.83777:72 577.13629:682	Procyanidin B2
B) Glycosides							
11.	1.148	611.1952	230851	1.6	$C_{28}H_{36}O_{15}$	265.09213:290 323.02719:73 323.05258:484 611.13629:865	Neohesperidin dihydrochalcone
12.	5.600	449.1079	77410	-10	$C_{21}H_{22}O_{11}$	449.08392:72 449.10785:287	Okanin 4'-*O*-glucoside
13.	6.620	463.089	2169458	-0.7	$C_{21}H_{20}O_{12}$	218.95721:214, 286.93921:286 300.02661:1164,301.04016:8193 54.91953:288 463.08578:3075	Quercetin 4'-glucoside
14.	1.382	591.0759	33049	-0.4	$C_{28}H_{32}O_{14}$	248.96024:214 591.09955:107	Acacetin 7-*O*-rutinoside
15.	3.705	449.1075	106326	-9.1	$C_{21}H_{22}O_{11}$	287.06189:71 449.10831:287	Eriodictyol 7-*O*-glucoside
16.	4.565	445.1363	510192	-6.4	$C_{21}H_{18}O_{11}$	197.04639:214 265.08951:107 445.13654:1144	Baicalein 7-*O*-glucuronide
17.	4.972	609.1481	58316	-2.1	$C_{27}H_{30}O_{16}$	609.14807:257	Luteolin 7,3'-di-*O*-glucoside
18.	5.046	577.1909	375177	0.9	$C_{27}H_{30}O_{14}$	489.21844:293 531.17761:71 577.19812:985	Rhoifolin
19.	6.413	593.2265	40943	-2.3	$C_{28}H_{34}O_{14}$	549.23199:107 575.28552:73 593.15045:222 593.1814:334 593.22266:343	Isosakuranetin 7-*O*-neohesperidoside
20.	7.253	595.1995	166714	3.9	$C_{27}H_{32}O_{15}$	549.19983:290 595.18231:660	Eriodyctiol 7-*O*-neohesperidoside
21.	7.755	433.1153	161404	-1.1	$C_{21}H_{22}O_{10}$	271.05899:364	Naringenin 7-*O*-glucoside
22.	8.164	593.1895	253433	-33.9	$C_{27}H_{30}O_{15}$	465.16193:71 487.15652:107 501.19513:71 503.17935:250 531.11633:73 547.17328:481 557.625:75 593.1861:1138	Kaempferol 7-neohesperidoside
23.	1.112	463.0599	65647	0	$C_{21}H_{20}O_{12}$	191.06053:107 249.02081:107 427.12738:107 463.05969:250	Myricitrin
24.	5.650	625.1371	63122	5.2	$C_{27}H_{30}O_{17}$	625.12646:296 625.13708:593	Quercetin3,4'-*O*-di-β-glucopyranoside

25.	6.914	593.1532	1878903	-1.7	$C_{30}H_{26}O_{13}$	283.11972:73 284.03528:328 285.04053:736 593.14978:3392	Kaempferol3-*O*-(6-p-coumaroyl)-glucoside
26.	7.333	447.0936	13787302	-0.6	$C_{21}H_{20}O_{11}$	`151.0016:214 178.9988:157 255.03139:337,271.0227:595 300.0301:3206,301.0338:3382 445.6438:286,447.09381:5592	Quercetin 3-*O*-α-L-rhamnoside (Quercitrin)
27.	8.084	431.099	2687403	-0.7	$C_{21}H_{20}O_{10}$	227.03609:179 243.02875:72 255.03072:396 278.91812:74 284.02853:453 285.04092:1618 431.09338:2245	Kaempferol 3-*O*-α-L-rhamnoside (afzelin)
28.	8.176	461.1075	19247	6	$C_{21}H_{18}O_{12}$	256.9361:71 324.90826:179 383.07071:72 392.90363:325 460.87732:214 461.10776:250	Kaempferol 3-glucuronide
29.	8.013	623.1964	20895	1.7	$C_{28}H_{32}O_{16}$	577.20837:119 623.19702:143	Isorhamnetin 3-*O*-rutinoside
30.	8.298	433.1486	37029	-0.8	$C_{20}H_{18}O_{11}$	296.92169:71 364.90253:179 433.11356:179 433.13708:220	Quercetin 3-D-xyloside
31.	17.081	477.1834	216770	4.9	$C_{22}H_{22}O_{12}$	477.18695:143	Isorhamnetin 3-*O*-glucoside
32.	5.441	415.1576	160818	6.6	$C_{21}H_{20}O_{9}$	269.10501:143 415.16074:250	Daidzein 8-*C*-glucoside
33.	6.305	609.1476 $[M–2H]^-$	5636360	-1.2	$C_{27}H_{31}O_{16}$	272.034:74 300.02911:454 301.04263:596 563.21997:71 609.14294:4929	Delphinidin 3-*O*-β-rutinoside
Phenolic acid derivatives							
34.	1.222	153.015	77803	13.8	$C_{7}H_{6}O_{4}$	109.02404:219 109.02846:367 153.02077:214	3,4-Dihydroxybenzoic acid
35.	1.357	353.0869	249581	1.1	$C_{16}H_{18}O_{9}$	173.04253:436 173.09084:107 175.09573:73 179.03056:395 191.05447:1028 353.0816:645	Chlorogenic acid
36.	2.778	167.0336	178872	5.2	$C_{8}H_{8}O_{4}$	123.04467:170 167.03207:337	Homogenentisic acid
37.	3.644	359.0984	108038	5.2	$C_{18}H_{16}O_{8}$	197.04456:86 359.09076:358	Rosmarinic acid
38.	4.357	137.0236	1463858	1.6	$C_{7}H_{6}O_{3}$	108.01912:143 119.01611:108 136.0172:364 137.02371:2252	*p*-Hydroxybenzoic acid
39.	6.876	167.0352	1497431	-0.7	$C_{8}H_{8}O_{4}$	108.02086:475, 124.01628:250 152.01102:733 167.03387:2224	5-Methoxysalicylic acid
40.	1.406	163.0514	39464	-6.2	$C_{9}H_{8}O_{3}$	119.04873:107, 119.05027:107 163.03922:71	3-(4-Hydroxyphenyl) prop-2-enoic acid
41.	4.838	183.0318	30577	-7.4	$C_{8}H_{8}O_{5}$	183.02837:250	3,4-Dihydroxymandelic acid
42.	8.568	179.0335	17409	0	$C_{9}H_{8}O_{4}$	179.03819:71 179.04008:143	Caffeic acid
43.	10.824	207.0659	15056	0	$C_{11}H_{12}O_{4}$	192.04141:71 207.06432:71	Sinapyl aldehyde
44.	1.222	385.1147	143072	-0.8	$C_{17}H_{22}O_{10}$	112.98918:143 248.96461:322 385.11237:478	1-*O*-β-D-Glucopyranosyl sinapate
Coumarins							
45.	2.778	191.0344	147298	0	$C_{10}H_{8}O_{4}$	120.0248:107 148.01596:250 176.01279:940 191.03516:434	Scopoletin
46.	7.547616	177.0179	126813	0	$C_{9}H_{6}O_{4}$	149.0222,177.0182:507	6,7-Dihydroxy-coumarin, esculetin
47.	3.967	339.2019	259023	-1.3	$C_{15}H_{16}O_{9}$	339.19983:716	Esculin
Organic acids							
48.	1.062	161.0447	154111	0.8	$C_{6}H_{10}O_{5}$	87.04898:72 99.04797:181 143.03127:71 161.04741:250	3-Hydroxy-3-methylglutaric acid
49.	1.087	133.0134	3183124	1.3	$C_{4}H_{6}O_{5}$	71.01123:901 72.99318:400 89.02149:145 89.02548:364 115.0032:2020,133.0133:1771	D- (+)-Malic acid
50.	1.124	209.0645	129423	4.7	$C_{6}H_{10}O_{8}$	85.02794:71 209.05681:143 209.06293:250	Mucic acid

51.	1.136	191.0561	12263925	-0.5	$C_7H_{12}O_6$	58.00823:217, 85.03046:2091 87.00948:412, 93.03408:765 109.03312:219 111.04481:259 127.03997:937 137.0258:214, 171.0237:255 173.04636:679 190.44801:286 191.05655:8643	D-(-)-Quinic acid
52.	1.136	165.0393	1033171	2.2	$C_9H_{10}O_3$	75.00899:331 147.03482:145 165.03989:896	D-3-Phenyllactic acid
53.	1.197	103.0405	419420	-1	$C_4H_8O_3$	57.03574:328 103.03818:519	Hydroxy butyric acid
54.	1.357	147.0291	189137	0.2	$C_5H_8O_5$	147.06203:107	Citramalic acid
55.	1.369	193.0719	213464	-0.5	$C_6H_{10}O_7$	161.04732:71 193.0603:143	D-(+)-Galacturonic acid
56.	1.432	175.0971	617021	0.4	$C_7H_{12}O_5$	101.05961:71 129.05035:143 131.06992:73 132.25606:72 157.08662:73 175.0997:479	2-Isopropylmalic acid
57.	1.694	190.9565	135104	-6.4	$C_6H_8O_7$	191.05638:400	Isocitric acid
58.	2.188	182.0453	35750	-2.1	$C_8H_9NO_4$	138.05557:71 182.04726:71	4-Pyridoxic acid
59.	4.287	173.116	28248	0	$C_8H_{14}O_4$	173.11824:134	Suberic acid
60.	6.681	407.1891	34542	-0.1	$C_{24}H_{40}O_5$	407.08401:107 407.08688:107	Cholic acid
Purines and pyrimidine derivatives							
61.	1.075	347.0618	567056	-4.7	$C_{10}H_{13}N_4O_8P$	115.00018:214 132.59344:219 133.01331:1326 191.05009:437 347.05692:1254	Inosine-5'-monophosphate
62.	1.087	321.0426	68749	0.2	$C_{10}H_{15}N_2O_8P$	133.01228:179 175.02496:71 321.02786:107 321.03799:179	Thymidine-5'-monophosphate
63.	1.357	241.1037	66700	12.5	$C_{10}H_{14}N_2O_5$	197.14574:71 241.14406:71 241.15283:71	Thymidine
64.	1.457	363.0943	87805	0.9	$C_{10}H_{13}N_4O_9P$	227.06593:107 257.08493:71 363.08899:214	Xanthosine-5'-monophosphate
65.	1.756	243.0615	38278	0.6	$C_9H_{12}N_2O_6$	110.02168:71, 140.03403:71 175.09375:71 200.05898:71 243.05753:71, 243.07735:71 243.11919:71 243.12579:71	Uridine
66.	1.793	134.0462	437357	1.8	$C_5H_5N_5$	107.03094:73 107.03386:254 134.04669:1164	Adenine
67.	2.139	549.2001	99915	-1.2	$C_{15}H_{24}N_2O_{16}P_2$	505.20883:71 549.19684:250	UDP-β-L-rhamnose
68.	4.565	467.1156	68903	0.6	$C_9H_{15}N_2O_{14}P_3$	467.11575:214	2'-Deoxyuridine-5'-triphosphate sodium salt
69.	5.798	307.103	5058	-1	$C_9H_{13}N_2O$	102.95472:71 307.07581:107	2'-Deoxyuridine-5'-monophosphate
70.	6.389	227.1273	17590	0.5	$C_9H_{12}N_2O_5$	209.11374:71 227.12993:214	2'-Deoxyuridine
71.	7.767	535.1783	68159	-4.7	$C_{14}H_{22}N_2O_{16}P_2$	535.13:72 535.18225:250	UDP-xylose
72.	9.326	604.2082	17919	-5.3	$C_{16}H_{25}N_5O_{16}P_2$	558.23499:72 604.18738:180167	Guanosine-5'-diphosphoglucose sodium salt
73.	10.432	202.0504	39411	-1.5	$C_{10}H_{13}N_5$	202.04362:107 202.05367:214	Isopentenyladenine
74.	12.631	579.2016	11493	10.7	$C_{15}H_{22}N_2O_{18}P_2$	579.18512:89	Uridine 5'-diphosphoglucuronic acid
75.	9.891	455.1712	24505	0.1	$C_{17}H_{21}N_4O_9P$	455.16489:150	Riboflavin5′-monophosphate sodium salt hydrate
76.	1.320	151.0615	356560	-1.6	$C_5H_4N_4O_2$	59.01287:107 151.06734:179	Oxypurinol
77.	1.457	304.1012	27159	-2.9	$C_9H_{12}N_3O_7P$	304.0968:71 304.10172:71 304.10419:71	Cytidine-3',5'-cyclicmonophosphate
Amino acids derivatives							

78.	7.162	174.0578	146587	-4.1	$C_6H_9NO_5$	128.05583:71 172.05193:72 174.05455:437	N-Acetylaspartic acid
79.	1.173	146.0445	21709	0	$C_5H_9NO_4$	102.05996:71 146.04488:71	DL-Threo-β-methylaspartic acid
80.	8.176	144.0463	455147	-4.7	$C_7H_{15}NO_2$	144.04683:1493	L-β-Homoisoleucine
81.	5.945	225.1138	45178	1	$C_9H_{14}N_4O_3$	225.10616:143	Carnosine
82.	1.332	129.0176	77352	8.9	$C_6H_{10}O_3$	129.02129:107	Ketoisoleucine
83.	1.160	128.0347	1421061	0.2	$C_5H_7NO_3$	128.03522:1007	L-5-Oxoproline
Sugars and polyhydric alcohols derivatives							
84.	1.320	181.0709	248306	0.7	$C_6H_{14}O_6$	83.0122:71 89.02168:107 163.06612:71 181.06931:250	Galactitol
85.	1.332	179.0546	3558333	5.3	$C_6H_{12}O_6$	58.00911:226, 59.01371:1809 71.0138:1105, 89.02305:939 101.02095:143 113.02514:322 161.04378:286 179.05696:329	D-(+)-Galactose
86.	1.332	341.1081	4380951	-0.1	$C_{12}H_{22}O_{11}$	59.01498:143 89.02455:220 113.02529:179 119.03493:214, 161.04207:250, 176.05005:74 179.04192:181 179.05325:751 340.0087:154 341.10849:1634	Sucrose
87.	1.432	243.0594	51063	7.3	$C_6H_{13}O_8P$	175.10318:107 207.10304:107 243.07527:109 243.1237:181	α-L-(-)-Fucose 1-phosphate bis(cyclohexylammonium) salt
88.	1.494	358.0131	27039	-3	$C_{10}H_{17}NO_9S_2$	358.0104:1074, 358.06384:293 359.0800:586,359.12015:2149 360.1271:1074, 361.10593:488 362.04041:488, 358.11496:107	2-propenylglucosinolate
89.	4.029	261.131	11930	1.4	$C_6H_{15}O_9P$	187.09714:71 261.12906:60 261.13589:60	Sorbitol 6-phosphate
90.	6.510	195.0329	84576	0	$C_6H_{12}O_7$	195.03336:286	Gluconic acid
91.	7.464	341.0664	70794	0.7	$C_{12}H_{22}O_{11}$	231.03505:71 341.0719:439	D-(+)-Trehalose
Others							
92.	1.087	405.0976	781550	8.3	$C_{20}H_{22}O_9$	191.054:997 265.09369:71 403.80188:77 405.09695:2317	*E*-3,4,5'-Trihydroxy-3'-glucopyranosyl-stilbene
93.	4.752	391.1216	267135	3.8	$C_{24}H_{40}O_4$	391.12753:179	Sodium deoxycholate
94.	5.663	381.1786	49906	-3	$C_{17}H_{35}O_7P$	381.18201:179	1-Myristoyl-2-hydroxy-sn-glycero-3-phosphate
95.	6.669	135.0422	19863	0	$C_{10}H_{16}$	135.04263:71 135.04591:178 135.04755:71 135.04919:107	γ-Terpinene
96.	8.359	138.0192	663498	0.8	$C_6H_5NO_3$	108.02113:325 138.01791:1303	*p*-Nitrophenol
97.	11.333	665.177	23872	-12.3	$C_{24}H_{42}O_{21}$	529.20654:179, 596.86041:179 619.39459:74 664.84143:259 665.17645:214	Nystose
98.	19.086	345.2051	269698	1	$C_{19}H_{22}O_6$	277.21567:214 345.19434:323	Gibberelin A3
99.	20.045	205.1582	33303	5.5	$C_2H_8O_7P_2$	205.15927:369	Etidronic acid

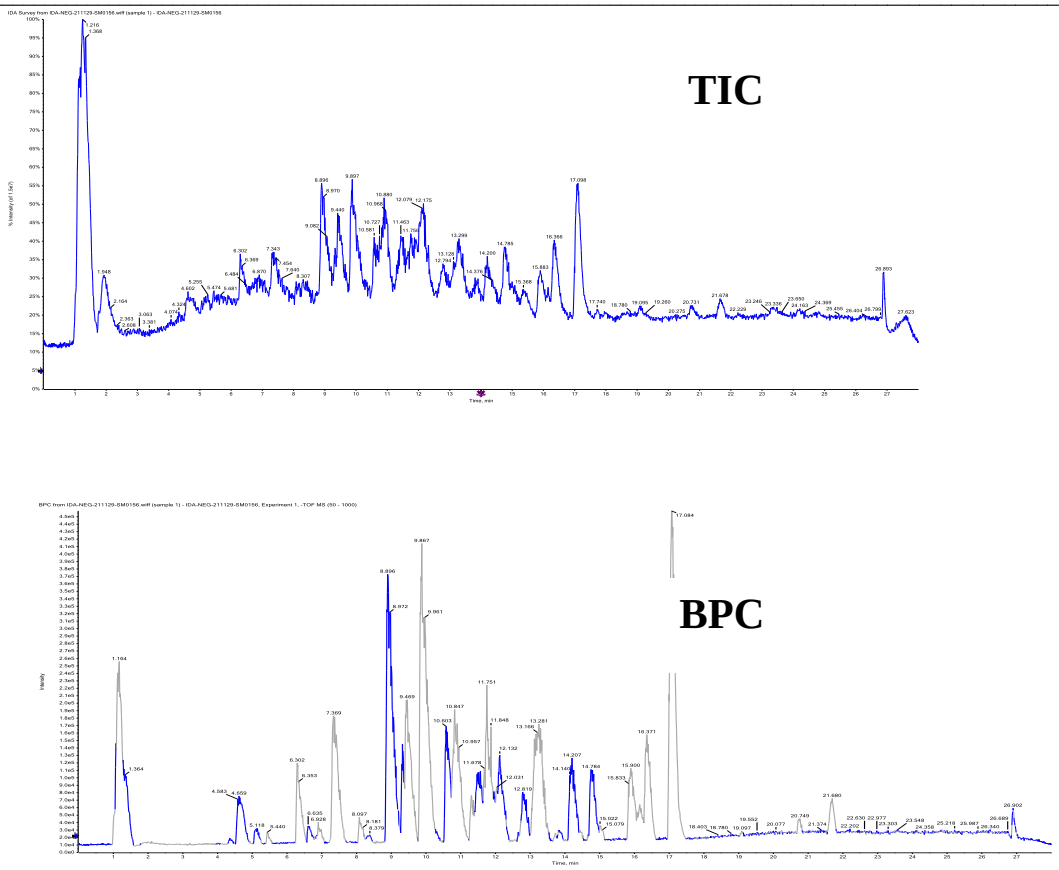

Figure 1. Negative ion mode TIC and BPC MS chromatograms of UPLC-ESI-qTOF-HRMS/MS for the MSP of the *K. grandfoliola* aerial parts extract (Peak numbers agree with those in table 3).

3.3.1. DPPH Radical scavenging

Figure 2 showed antioxidant activity of the total and MeOH-soluble portions (MSP) of *K. senegalensis* and *K. grandfoliola* using the DPPH assay. All the examined extracts recorded good antioxidant activity. The total extract of *K. senegalensis* had superiority among the other studied extracts; it recorded 73.83±0.20%, followed by the MSP (71.67±0.39%). Lower DPPH radical scavenging activity was recorded with the total and methanol extracts of *K. grandfoliola*; it was achieved to be 65.43±0.29 and 63.30±0.27%, respectively, at a concentration of 250 µg/ml. Standard synthetic antioxidant BHA radical scavenging activity was found to be 91.44±0.29% at 100 µg/ml. Similar results reported the antioxidant activity of *K. senegalensis* leaf extract, using the DPPH assay, as 42.69% [54], which was higher than *K. senegalensis* bark extract (36.36%). This is highly consistent with other studies that investigated the aqueous leaves extract of *K. senegalensis* showing a reduction in DPPH with IC_{50} of 44.88 ± 0.43 µg/ml [55].

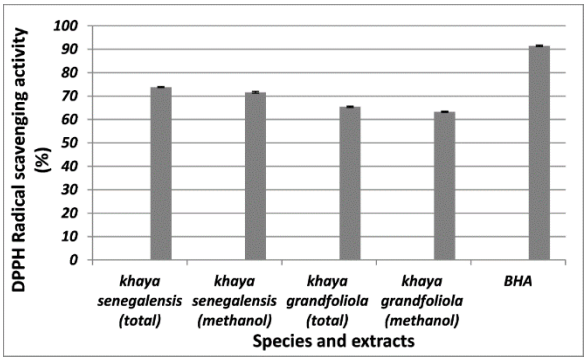

Figure 2. DPPH Scavenging inhibition (%) of the total extract and MSP (250 µg /ml) of *K. senegalensis* and *K. grandfoliola*. Values are means of three replicates ± Standard deviation.

3.3.2. Reducing power capability

As illustrated in figure 3, the extracts from the two *Khaya* species exhibited high reducing power capabilities in comparison withthe standard antioxidant BHA (at a lower concentration), which reinforces and affirms the high activity of all investigated extracts. Total extract of *K. senegalensis* recorded 0.958±0.03 (absorbance at 700 nm), while the MSP absorbance was found to be 0.880±0.01. On the other hand, *K. grandfoliola* reducing power ability was 0.722±0.01 and 0.672±0.01 for the total extract and MSP, respectively, at a concentration of 20 µg/ml.

The standard antioxidant BHA recorded 0.975±0.01 at the concentration of 100 µg/ml. Also, it was discovered that the extract of *K. grandifoliola* has a reducing power ranging from 0.1 to 1.5 (absorbance at 700 nm) at doses ranging from 0.1 to 100 µg/ml [56].

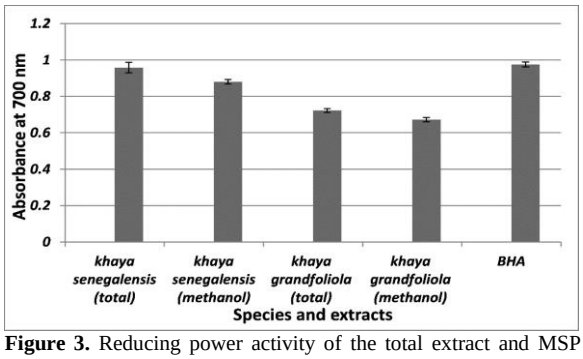

Figure 3. Reducing power activity of the total extract and MSP (20µg/ml) of K. senegalensis and K. grandfoliola. Values are means of three replicates ± Standard deviation.

3.3.3. Ferric reducing antioxidant power

The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe3+-TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe2+-TPTZ). The free radical chain breaking takes place by donating a hydrogen atom. Ferric reducing antioxidant power takes place in the same manner as the previous antioxidant methods. Thus, the total extract of *K. senegalensis* resulted in the highest FRAP; it was found to have 1784±10.82 µmol Trolox/100 g at a concentration of 20 µg/ml, followed by the MSP activity (1682±10.15 µmol Trolox/100 g) (Fig. 4). The FRAP recorded by the total extract and MSP of *K. grandfoliola* was lower but still reflected high FRAP power. Current results are in good agreement with those recorded by Marius *et al*. [57]. They found that all the examined extracts and fractions possess high FRAP values ranging from 13.04±0.25 to 13.60±0.09 mmol Trolox equivalent/g (mmol TE g^{-1}) of extract or fraction of *K. senegalensis* A. juss. (Meliaceae) stem barks.

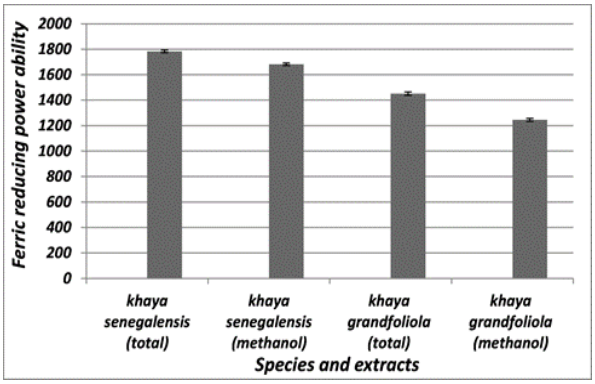

Figure 4. Ferric reducing power ability of the total extract and MSP (20µg/ml) of *K. senegalensis* and *K. grandfoliola.* Values are means of three replicates ± Standard deviation.

3.3.4. ABTS Radical scavenging capacity

ABTS scavenging radical capacity revealed that *K. senegalensis* possesses very high ABTS radical scavenging capacity by recording 86.19±0.34 and 70.02±0.21% compared to 50.48±0.16 and 39.72±0.36% for the total extract and MSP of *K. grandfoliola* at 20µg/ml. The recorded activity is considered very strong, even more than the antioxidant activity of the synthetic antioxidant standard BHA, which was found to be 88.42±0.24% at 100µg/ml (Fig. 5). In a similar way, a previous study [57] reported that the antioxidant activity of *K. senegalensis* A. juss. stem bark was determined using the ABTS•+ radical scavenging activity of aqueous ethanol extract and fractions [57]. The results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) values, which ranged from 8478±0.3 to 3±0.05. The *n*-hexane fraction showed the highest capacity to scavenge ABTS•+.

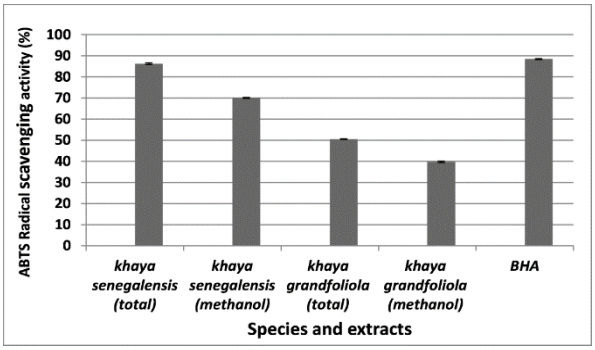

Figure 5. ABTS Radical scavenging activity (%) of the total extract and MSP (20µg/ml) of K. senegalen-sis and K. grandfoliola. Values are means of three replicates ± Standard deviation.

It is clearly noticeable that the antioxidant activity of the total extract and MSP of *K. senegalensis* was constantly promising with all assays.

3.4. Effect on the viability of EACC

The current investigation is focused on *K. senegalensis* and *K. grandfoliola* extracts, preliminary anticancer efficacy, tumor-specific action, and characterization of the active ingredients in total extract and MSP of *Khaya* species which are responsible for this action. Previous reports [58] reported that Ehrlich Ascites Carcinoma (EAC) cells have a great potential for transplantation, no regression, extremely rapid proliferation, a shortened lifespan, 100% malignancy, and no tumor-specific transplantation antigen. Figure 6 clearly indicates that the total extract and MSP of *K. senegalensis* and *K. grandfoliola* exhibited a high effect on the viability of EAC cells using different concentrations (10, 25, 50 and 100 µg/ml). *K. senegalensis* total extract reduced the viability of EAC cells in a dose-dependent manner to (26.54±0.30, 44.51±0.23, 66.28±0.14, and 88.39±0.22) dead cells % for 10, 25, 50, and 100

µg/ml, respectively. Comparatively, *K. grandifoliola* total extract caused lower percentage inhibition at the same concentrations; for example, 100 µg/ml recorded 71.64±0.40 dead cells%. Concerning the MSP of *K. senegalensis* and *K. grandfoliola*, they achieved 82.68±0.26 and 66.54±0.23 at high concentrations (100 µg/ml). It is clearly noticeable from the results that the high antitumor activity of *K. senegalensis* total extract was found to be as close as that of the standard drug vincristine, which achieved 90.64±0.39% dead cells at the same concentration (100 µg/ml). All the previous results can be summarized by saying that *K. senegalensis* total and MSP are highly active antioxidant and antitumor agents, which prompted us to isolate and identify the active pure compounds and evaluate them biologically in other current study.

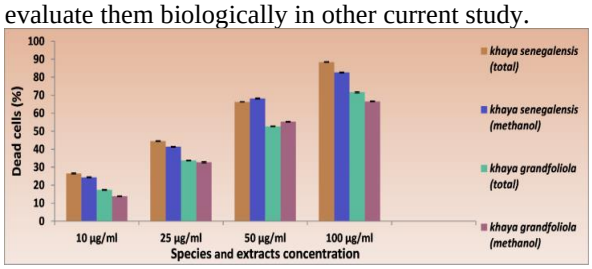

Figure 6. Effect of different concentrations of the total extract and MSP of K. senegalensis and K. grandfoliola on the viability of EACC. Values are averages for 3 independent experiments and standard deviation.

4. Discussion

Qualitatively, it can be concluded that the results of preliminary phytochemical screening (Table 2) are in good agreement with the results found by LC-MS/MS (Table 3). Although the good separation shown by time-differences among the MS peaks in both TIC and BPC mass chromatograms (Fig. 1), the coelution of more than one metabolite in each peak was concluded because of the lower number of peaks relative to the number of identified and unidentified metabolites from the MSP of *K. grandifoliola*. XIC chromatograms and MS/MS spectra outlined the relative concentration (peak area) and structural characters (certain fragment/s) for each constitutive metabolite in the investigated extract (Figs. 1S–99S). In addition, the fragmentation pattern can be followed up and explained for interpretation and identification of the constitutive metabolites although the identification is based mainly on the blind matching of the experimental data with the library database and literature. So, the identification by such techniques, in most cases, is quite enough for confirmation of the accurate stereo structures, i.e. structural, configurational, or conformational isomerism, because of the various parameters already included in the stored library (see section 2.5) [48,49,52]. This may be explained by the accompanying chemical names of the identified metabolites with some of their stereochemical features (e.g.*α/β-*, *R/S-*, *E/Z*-isomers) [39,59–63]. The structural information, even stereochemical characters, depend on the formation or absence of certain selective fragment ion/s and/or the extent of the relative abundances of the monoisotopic molecular or fragment ion/s. It is worthy that the fragmentation pattern and some specific fragment ion/s are directly correlated to the type of the chemical structure for each class of the investigated metabolites. In case of flavonoid aglycones (**1**–**10**), they gave their molecular ions as a base peak (Figs. 1S–10S) accompanied with a low number of fragments by the loss of H, OH, CO or through the specific Retro-Diels-Alder cleavage (RDA) of the ring C [64]. For instance, myricetin (**1**), isorhamnetin (**2**), quercetin (**3**), kaempferide (**4**), naringenin (**6**) and luteolin (**9**) showed the corresponding XIC chromatograms and the molecular ions [M–H]– in their MS^2 spectra as the base peaks at m/z 317.0514, 315.1071, 301.0361, 299.0554, 271.0600 and 285.0419, respectively. Unlike aglycones, at high CID potential energy the flavonoid O-glycosides (**11**–**33**) start to cleave step-wisely from outer to inner the glycoside moiety up to the corresponding aglycone/s that are further cleaving according to RDA-fragmentation pattern of C-ring. Normally the extent of degradation energy required for cleaving C-glycosides will be higher than that enough energy for starting the cleavage of O-glycosides, but lower than the degradation energy of the free aglycones. Accordingly, quercitrin (**26**, Rt= 7.333, area= 13787302) and delphinidin 3-O-β-rutinoside (**33**, R_t= 6.305, area= 5636360) are noticed as the most prominent pair of O-glycosides in the extract (Table 3). The first demonstrated a molecular ion at m/z 447.0929 $[M-H]^-$ and aglycone ion at m/z 301.0361 $[M-H-146]^-$, with its oxidative form at 300.0281 $[M-2H-146]^-$, calcd. for 447.2222, 301.0311, and 300.0258, corresponding to the loss of rhamnosyl moiety (Fig. 26S, Table 3). Another characteristic oxidative fragment from the aglycone after the loss of CO molecule from ring C was observed at 271.0233 $[M\text{-rhamnosyl-CO-3H}]^-$ in the MS^2-spectrum, calcd. for 271.01987. It is worthy that the second major O-glycoside represented only one anthocyanin-type flavonoid in this plant extract. It shows an oxidative molecular ion at m/z 609.1448 $[M-2H]^-$, because of its ready positively charged form in nature. It is calculated as 609.1456 for MF $C_{27}H_{31}O_{16}$ and MW 611.1612 (Fig. 33S, Table 3). At high CID fragmentation energy, it produced a characteristic aglycone fragment at m/z 301.0335 $[M-2H-308]^-$ corresponding to the loss of rutinoside moiety together with an oxidative fragment at 300.0290 $[M-3H-308]^-$ (Table 3). The identification of both metabolites based automatically on the matching with the library database and further reinforced by previously reported data [65,66] Like data was observed for the kaempferol analogue of 26 as the next major metabolite, where kaempferol 3-O-α–L-rhamnoside (afzelin, **27**, R_t= 8.084, area= 2687403, Table 3) showed a molecular ion peak at m/z 431.0972 $[M-H]^-$calcd. for 431.1907 amu in its MS^2 spectrum (Fig. 27S). A typical aglycone fragment was recorded at m/z

285.0408 [M-H-146]$^-$ followed by oxidative product ion at 284.0318 [M-2H-146]$^-$ due to the loss of rhamnosyl moiety. This library database-based identification was in good accordance with the literature of afzelin [67]. At high CID fragmentation energy another O-glycoside metabolite (**13**), displayed a molecular ion at m/z 463.0861 [M-H]$^-$ together with characteristic aglycone fragment ions at m/z 301.0367 [M-H-162]$^-$ and its oxidative ion at 300.0273 [M-2H-162]$^-$ in MS^2 spectrum (Fig. 13S), due to the loss of the glucoside moiety. Such peaks were in good consistency with the calcd. ones in the library database at 463.09052, 301.0434, and 300.02493 (Table 3)and agreed with the MF $C_{21}H_{20}O_{12}$ of quercetin 4'-O-glucoside [65]. Also, okanin 4'-O-glucoside (marein) showed a molecular ion peak [M–H]$^-$ at m/z 449.1074 (calcd. 449.1084 for MF $C_{21}H_{22}O_{11}$ and MW 450.1162) in its MS^2 spectrum (Fig 12S, Table 3) with an aglycone ion at m/z 287.0593 [M-H-162]$^-$ corresponding to the loss of glucose moiety. This was in good matching with the library database and reported literature [66]. Moreover, an acylated kaempferol 3-glucoside revealed a molecular ion peak at m/z 593.1468 [M-H]$^-$, and aglycone fragment ion at m/z 285.0401 [M-H-308], calcd. for 593.1535 and 285.0433 amu, respectively (Fig. 25S). According to the mentioned documents and matching with the library database and literature [68], it is identified as kaempferol 3-O-(6-*p*-coumaroyl)-glucoside.

As another good example for the high efficacy of negative HRESI-MS^2 for identification of plant polyphenols, 11 phenolic acids (**34**–**44**) were unambiguously identified (Table 3). Commonly, the deprotonated molecular ion ([M-H]$^-$) acted as the precursor for some of the characteristic fragment ions in MS^2 spectra such as decarboxylation (-CO_2, 44 amu) and dehydration (-H_2O, 18 amu) product fragments in case of acids (Figs. 34S–44S). Thus, at high CID potential, the MS^2 spectrum of 3,4-dihydroxybenzoic acid (protocatechuic acid, **34**) displayed a molecular ion at m/z 153.0228 [M-H]$^-$, calcd. 153.0188 for MF $C_7H_6O_4$ and MW 154.0266, (Fig. 34S, Table 3). In addition, a characteristic product ion at m/z 109.0292 [M-H-44]$^-$, corresponding to the decarboxylation (–CO_2) [69]. Figure 35S clarify the XIC and MS^2 of 3-O-caffeoylquinic acid (chlorogenic acid, **35**) displayed a molecular ion at m/z 353.0865 [M-H]$^-$ calcd. 353.0873 for MF $C_{16}H_{18}O_9$ and MW 354.0951. Further characteristic product ions at m/z 191.0562 [M-H-162]$^-$ corresponding to [quinate]$^-$ or the loss of caffeoyl, 179.0376 [M-H-173]$^-$ corresponding to [caffeate]$^-$ and 135.0443 [caffeate-CO_2]$^-$[70]. The fragmentation pattern differentiated the two C-skeleton isomers, i.e. homogentisic (**36**) and 5-methoxysalicylic (**39**) acids. Both gave a molecular ion peak at m/z 167.0347 or 167.0354 [M-H]$^-$, calcd. 167.0344 for MF $C_8H_8O_4$ and MW 168.0423, (Figs. 36S, 39S, Table 3). However, at high CID potential 36 showed fragment ions at 149.0205 [M-H-18]$^-$ and 123.0449 [M-H-44]$^-$, corresponding to dehydration and decarboxylation, while **39** gave fragments at 152.0123 [M-H-15]$^-$ and 108.0187 [M-H-15-44]$^-$ corresponding to the loss of CH_3 only then CH_3 and CO_2 due to the presence of the OMe group in the structure of the second [71]. Similarly, rosmarinic acid gave rise a [M-H]$^-$ ion at m/z 359.0971, calcd. 359.0767 for MF $C_{18}H_{16}O_8$ and MW 360.0845, (Fig. 37S), together with a main product ion at m/z 197.0458 [M-162]$^-$ due to the loss of caffeoyl moiety among other characteristic fragments (Table 3) [72]. The remaining phenolic acids obeyed in their MS^2 spectra (Fig. 38S, 40S–44S) the same fragmentation pattern, including a base molecular ion peak with degradation of OH, CH_3, dehydration or decarboxylation. Concerning the third phenolic-type metabolites identified in MSP of *K. grandifoliola*, three 6,7-dihydroxycoumarin derivatives were detected, i.e. scopoletin (**45**), esculetin (**46**) and esculin (**47**). At high CID the coumarins release characteristic fragments by successive loss of CO and /or CO_2 and or H_2O according to matching with published data and library database [73–75]. Scopoletin displayed a deprotonated ion at m/z 191.0344, calcd. 191.0344 for MF $C_{10}H_8O_4$ and MW 192.0423, (Fig. 45S), alongside two main characteristic product ions at m/z 176.0112 [M-H-CH_3]$^-$, and 148.0169 [M-H-CH_3-CO]$^-$. Coumarin 46 (esculetin) exhibited a molecular ion peak [M-H]$^-$ at m/z 177.0192, calcd. 177.0190 for MF $C_9H_6O_4$ and MW 178.0266, and two fragment ions at m/z 149.0222 [M-H-28]$^-$ and 133.0319 [M-H-44]$^-$ corresponding to the loss of CO and CO_2 neutral molecules. Furthermore, esculetin 6-O-glucoside (esculin, **47**) revealed a molecular ion peak at m/z 339.2005 [M-H]$^-$ calcd. 339.0716 for MF $C_{15}H_{16}O_9$ and MW 340.0794 (Fig. 47S). The structures of all other identified metabolites according to the blind matching of their output MS/MS data with the corresponding library database can be followed and explained according to their published data and fragmentation pattern as it was discussed above for polyphenols.

Antioxidants break down peroxides, scavenge radicals, and start chains to shield cells from the damaging effects of reactive oxygen species [76]. Natural antioxidants reduce DNA damage, cell damage, malignant transformation, and the start of diseases including cancer, heart disease, and oxidative stress by scavenging free radicals. The strong antioxidant activity of the studied total extracts and MSP could be attributed and correlated to the high content of phenolic compounds, which serve as metal ion chelators, hydrogen-donating sources, free radical scavengers, and singlet oxygen quenchers [77]. Oxidative stress is the root cause of numerous illnesses, including diabetes, high blood pressure, and

atherosclerosis [78]. The antioxidant and cytotoxic effects of the extracts could be attributed to the high content of phenolic compounds (flavonoids, phenolic acids, coumarins, and anthocyanins) identified. The reactivity of the phenolic moiety with a strong radical scavenging activity via hydrogen atom donation makes phenolic metabolites recognized as direct antioxidants [79]. In the same direction, it is suggested that *K. grandifoliola* extract was found to include flavonoids, saponins, tannins, alkaloids, triterpenoids, reducing sugars, cardiac glycosides, and certain necessary elements like potassium and sodium [80]. The phytochemicals and elements found in plant extracts may have, if they worked in concert, produced the antimalarial, antioxidant, and anti-inflammatory benefits described in their study. The subsequent formation of more stable tannic radicals would have the effect of blocking the lipid autooxidation chain reaction [81]. Because of their low redox potentials, flavonoids possess the capacity to transfer hydrogen and reduce oxidizing free radicals such as superoxide, peroxyl, alkoxyl, and hydroxyl. The flavonoxy radical may subsequently interact with another radical to form a stable structure [82]. Moreover, it was suggested that the ability of coumarins to suppress ROS is correlated with the number of hydroxyl groups in their ring structure [83]. According to a previous report [84], two hydroxyl groups are present on esculetin, which may result in the highest affinity for the xanthine oxidase (XO) binding site, as exhibited by its benzene rings. The two most potent radical scavengers are esculetin and 4-methylesculetin, both of which have two hydroxyl groups on the benzene rings. The scavenging radicals of the methoxy-substituted compound scopoletin performed less well than esculetin. This may be because the radicals generated from esculetin and 4-methylesculetin have resonating structures that are particularly stable due to the resonating structures of *ortho*-quinone form. Compared to the other examined coumarins, esculetin and 4-methylesculetin had greater resonance structures. The extracts from antioxidant principles-containing plants demonstrated anticancer efficacy in experimental animals and cytotoxicity toward tumor cells, as well. Plant-derived products exhibit cytotoxic and anticancer action through either inducing apoptosis or inhibiting neovascularization [85].

5. Conclusion

UPLC/ESI-qTOF-HRMS/MS analysis proved to be an efficient and precise tool for the qualitative and quantitative identification of the constitutive metabolites of complex plant extracts. Therefore, the probable biological effects can be explained because of the synergistic effects of the constituents according to their structures and relative concentrations in the investigated samples. In this study it was proved that the different types of polyphenols constitute a ratio of 45.71% of the total represented peaks in the MS chromatograms, including flavonoids, phenolic acids and coumarins. From the obtained results, we can conclude that *K. senegalensis* and *grandfoliola* total extracts and MSP possess high antioxidant and cytotoxic or antitumor activities. The total extract outperforms the methanol-soluble portion (MSP) in both species. It can be explained such significant biological activities based on the high polyphenolic content and large number of flavonoids and phenolic acids identified in the sample. Therefore, *Khaya* species could be an excellent source for natural antioxidant and antitumor agents for medical and nutraceutical applications. Further studies may lead to their use as safe alternatives to natural antioxidant and antitumor drugs.

Authors' contribution

R.M.E., A.M.Y.M., H.A.A.T and M.M. conceived and designed the experiments, searched for information, M.M. interpreted and wrote the output LC-MS/MS data. R.M.E. and A.M.Y.M. performed the extraction, and chromatographic examination. R.M.E., H.A.A.T and M.M. drafted the original paper. H.A.A.T: Design, evaluate, elucidate, and write the biological evaluation part. All authors contributed to the manuscript: revision, reading, and approval the submitted version.

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Declaration of Interest

The authors declare that they have no known competing financial interests.

Ethical Approval

Authors hereby declared that the experimental protocol was approved by Port Saied University – Faculty of science - Departmental committee for Research and Ethics (PSU.Sci.7.). All rules were followed as well as specific national laws where applicable.

List of abbreviations

UPLC/ESI-qTOF-HRMS/MS: Ultra-performance Liquid Chromatography/Electrospray Ionization-Quadrupole Time-of-Flight-High-resolution Mass spectrometry/ Mass spectrometry; HRESI-MS: High-resolution Electrospray Ionization-Mass spectrometry; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; ABTS: (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)); Trolox:6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydrochromene-2-carboxylic acid, BHA: butylated hydroxyanisole.

6. References

[1] M.O. Oyedeji Amusa, R.D. Stewart, M. van der Bank, B.-E. van Wyk, A Taxonomic Review of South African Indigenous Meliaceae Using Molec-ular Systematics and Anatomical Data, Diversity 16 (2024) 113.

https://doi.org/10.3390/d16020113.
[2] D.J. Mabberley, C.M. Pannell, A.M. Sing, Meliac-eae, Flora Malesiana-Series 1, Spermatophyta 12 (1995) 1–407.
[3] F.A. Hashem, E.A. Aboutabl, S.S. El-Souda, A. Selim, K. Shaker, A.A. Maamoun, Composition of lipoidal matter and evaluation of hepatoprotective, cytotoxic, and antioxidant activities of *Khaya grandifoliola* C. DC. growing in Egypt, Egypt. Pharm. J. 13 (2014) 13.
[4] T.L. Olatunji, C.A. Odebunmi, A.E. Adetunji, Biological activities of limonoids in the Genus *Khaya* (Meliaceae): a review, Futur. J. Pharm. Sci. 7 (2021). https://doi.org/10.1186/s43094-021-00197-4.
[5] J.S. Youn, Y.-J. Kim, H.J. Na, H.R. Jung, C.K. Song, S.Y. Kang, J.Y. Kim, Antioxidant activity and contents of leaf extracts obtained from Dendro-panax morbifera LEV are dependent on the collec-ting season and extraction conditions, Food Sci. Biotechnol. 28 (2019) 201–207.
[6] M. Abdel Azizi, N.A. Christelle Nadia, Y. Cedric, G.N. Guy-Armand, N.N. Aboubakar Sidiki, T.N. Jemimah Sandra, T.D. Alex Kevin, V.K. Payne, *In Vitro* Antiplasmodial, Cytotoxicity, and Antioxi-dant Activities of *Lophira lanceolata* (Ochnaceae): A Cameroonian Plant Commonly Used to Treat Malaria, J. Trop. Med. 2023 (2023).
[7] S. Harpreet, P. Leena, P. Pradeep, Review on cancer and anticancerous properties of some medicinal plants, J. Med. Plants Res. 5 (2011) 1818–1835.
[8] F. Bray, M. Piñeros, Cancer patterns, trends and projections in Latin America and the Caribbean: a global context, Salud Publica Mex. 58 (2016) 104–117. https: //doi.org /10.21149/ spm. v58i2. 7779.
[9] H. Wang, M. Naghavi, C. Allen, Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015, Lancet 388 (2016)1459–1544. https : //doi.org/https: //doi.org/10.1016/S0140-6736 (16) 31012-1.
[10] N. Tyagi, G.N. Sharma, B. Shrivastava, P. Saxena, N. Kumar, Medicinal plants: used in Anti-cancer treatment, Int. J. Res. Dev. Pharm. Life Sci. 6 (2017) 2732–2739.
[11] F.O. Ohiagu, P.C. Chikezie, C.M. Chikezie, C.E. Enyoh, Anticancer activity of Nigerian medicinal plants: a review, Futur. J. Pharm. Sci. 7 (2021) 70. https://doi.org/10.1186/s43094-021-00222-6.
[12] M. Harun-ur-R, M.A. Gafur, M.G. Sadik, M.A.A. Rahman, Biological Activities of a New Acrylamide Derivative from Ipomoea turpethum, Pakistan J. Biol. Sci. 5 (2002) 968–969. https://doi.org/ 10.3923/pjbs.2002.968.969.
[13] P.C. Chikezie, Comparative FT-IR analysis of chl-oroform fractions of leaf extracts of *Anacardium occidentale*, Psidium guajava and *Terminalia cat-appa*, J. Basic Pharmacol. Toxicol. 3 (2019) 1–6. https://doi.org/https://www.researchgate.net/publication/338114517.
[14] P.C. Chikezie, O.A. Ojiako, K.C. Nwufo, Overview of anti-diabetic medicinal plants: the Nigerian research experience, J Diabetes Metab 6 (2015) 546.
[15] B. Lawal, O.K. Shittu, F.I. Oibiokpa, E.B. Berinyuy, H. Mohammed, African natural products with potential antioxidants and hepatoprotectives properties: a review, Clin. Phytoscience 2 (2017) 23. https://doi.org/10.1186/s40816-016-0037-0.
[16] B. Lawal, O. Shittu, A. Kabiru, A. Jigam, M. Umar, E. Berinyuy, B. Alozieuwa, Potential antimalarials from African natural products: a review, J. Intercult. Ethnopharmacol. 4 (2015) 318. https://doi.org/10.5455/jice.20150928102856.
[17] L. Bashir, O. Shittu, S. Sani, M. Busari, K. Adeniyi, African Natural Products with Potential Antitrypanosomal Properties: A Review, Int. J. Biochem. Res. Rev. 7 (2015) 45–79. https://doi.org/10.9734/IJBCRR/2015/16039.
[18] I. Mapaure, T. Hatuikulipi, Namibia's Indigenous Knowledge System: IKS literature review report on the use of plants for medicinal and other purposes by local communities in Namibia, Univ. Namibia, Wind. (2008).
[19] D. Muhammed, O.B. Adaaja, I.G. Otiwa, Y.E. Zaman, O.E. Oloyede, Phyto-pharmacological and safety reports of *Khaya senegalensis* (Desr) A Juss (Meliaceae), an important multifunctional tropical forest plant, AROC Nat. Prod. Res. 01 (2021) 28–43. https://doi.org/10.53858/arocnpr01012843.
[20] S. Singh, D.B. Singh, S. Singh, R. Shukla, P.W. Ramteke, K. Misra, Exploring Medicinal Plant Legacy for Drug Discovery in Post-genomic Era, Proc. Natl. Acad. Sci. India Sect. B Biol. Sci. 89 (2019) 1141–1151. https://doi.org/10.1007/s40011-018-1013-x.
[21] A. Pledgie-Tracy, M.D. Sobolewski, N.E. Davidson, Sulforaphane induces cell type–specific apoptosis in human breast cancer cell lines, Mol. Cancer Ther. 6 (2007) 1013–1021. https://doi.org/10.1158/1535-7163.MCT-06-0494.
[22] A.L. Risinger, F.J. Giles, S.L. Mooberry, Microtubule dynamics as a target in oncology, Cancer Treat. Rev. 35 (2009) 255–261. https://doi.org/10.1016/j.ctrv.2008.11.001.
[23] M. Shoeb, Anticancer agents from medicinal plants, Bangladesh J. Pharmacol. 1 (2008) 35–41. https://doi.org/10.3329/bjp.v1i2.486.
[24] S. Sultana, H.M. Asif, H.M.I. Nazar, N. Akhtar,

J.U. Rehman, R.U. Rehman, Medicinal Plants Combating Against Cancer - a Green Anticancer Approach, Asian Pacific J. Cancer Prev. 15 (2014) 4385–4394. https://doi.org/10.7314/APJCP.2014.15.11.4385.

[25] M.-M. Zhou, W.-Y. Zhang, R.-J. Li, C. Guo, S.-S. Wei, X.-M. Tian, J. Luo, L.-Y. Kong, Anti-inflammatory activity of Khayandirobilide A from *Khaya senegalensis* via NF-κB, AP-1 and p38 MAPK/Nrf2/HO-1 signaling pathways in lipopo-lysaccharide-stimulated RAW 264.7 and BV-2 cells, Phytomedicine 42 (2018) 152–163. https://doi.org/10.1016/j.phymed.2018.03.016.

[26] I.L. Elisha, M.S. Makoshi, S. Makama, C.J. Dawurung, N. V Offiah, J.G. Gotep, O.O. Oladipo, D. Shamaki, Antidiarrheal evaluation of aqueous and ethanolic stem bark extracts of *Khaya senegal-ensis* A. Juss (Meliaceae) in albino rats, (2013). https://doi.org/http://www.pvj.com.pk/pdf-files/33_1/32-36.pdf.

[27] M. Osman Widdat Allah, Phytochemical screening, chemical composition and antioxidant activity of leaves and bark extracts from *Khaya senegalensis*, Adv. Biochem. 6 (2018) 32-38. https://doi.org/10.11648/j.ab.20180604.12.

[28] G.N. Guy-Armand, Y. Cedric, N.A. Christelle Nadia, M.A. Azizi, N.N. Aboubakar Sidiki, T.N. Jemimah Sandra, T.D. Alex Kevin, V.K. Payne, Antiplasmodial, Antioxidant and Cytotoxicity Activity of Ethanol and Aqueous Extracts of *Khaya grandifoliola* Stem Bark, J. Trop. Med. 2023 (2023).

[29] G.N. Guy-Armand, Y. Cedric, N.A.C. Nadia, T.D.A. Kevin, T.N.J. Sandra, N.N.A. Sidiki, M.A. Azizi, V.K. Payne, Efficacy of *Khaya grandifoliola* Stem Bark Ethanol Extract in the Treatment of Cerebral Malaria in Swiss albino Mice Using Plasmodium berghei NK65 Strain, J. Parasitol. Res. 2023 (2023).

[30] A. Falodun, R. Siraj, M.I. Qadir, S.A.K. Tanoli,M.I. Choudhary, Chemical composition and insec-ticidal activity of volatile oil of *Khaya grandifol-iola*, Med. Aromafic Plant Sci. Biotechnol. 3 (2009) 61–63.

[31] D.A. Tyoapine, P.C. Victory, A. Yusuf, A.M. Badamasi, A Review of the Phytochemistry and Pharmacology of the Medicinal Plant: *Khaya senegalensis* (Desr.) A. Juss, J. Homepage Www. Ijrpr. Com ISSN 2582 (2022) 7421.

[32] Y.X. Yan, J.Q. Liu, H.W. Wang, J.X. Chen, J.C. Chen, L. Chen, L. Zhou, M.H. Qiu, Identification and Antifeedant Activities of Limonoids from *Aza-dirachta indica*, Chem. Biodivers. 12 (2015) 1040–1046.https: //doi.org/10.1002/cbdv.201400282.

[33] S.A.M. Abdelgaleil, M. Nakatani, Antifeeding activity of limonoids from *Khaya senegalensis* (Meliaceae), J. Appl. Entomol. 127 (2003) 236–239. https://doi.org/10.1046/j.1439-0418.2003.00742.x.

[34] V.T. Hien, V.D. Hoang, Chemical constituents of fruits of *Khaya senegalensis* A. Juss growing in Vietnam, Vietnam J. Chem. 54(6) (2016) 781–785. https://doi.org/10.15625/0866-7144.2016-00404.

[35] C.M. Yuan, G.H. Tang, X.Y. Wang, Y. Zhang, F. Guo, J.H. Liao, T. Zou, G.Y. Zuo, H.M. Hua, H.P. He, X.J. Hao, Two new compounds from *Khaya senegalensis*, J. Asian Nat. Prod. Res. 15 (2013) 638–643. https://doi.org/10.1080/10286020.2013.794419.

[36] F. Hashem, E. Aboutabl, S. EL Souda, M. Moharr-am, A. Mammoun, M. Shabana, Desmutagenic and antimutagenic potential of phenolics from *Khaya grandifoliola* (C.DC.), Meliaceae, Egypt. Pharm. J. 12 (2013) 148. https://doi.org/10.4103/1687-4315.124018.

[37] H. Zhang, Characterization of bioactive phytoch-emicals from the stem bark of African Mahog-any *Khaya senegalensis* (Meliaceae), (2008).

[38] W.-Y. Zhang, L. Qiu, Q.-P. Lu, M.-M. Zhou, J. Luo, Y. Li, Furan fragment isomerized mexicanoli-de-type Limonoids from the stem barks of *Khaya senegalensis*, Phytochem. Lett. 24 (2018) 110–113.

[39] O. Kayser, P.M. Abreu, Antileishmania and immu-nostimulating activities of two dimeric proanthocy-anidins from *Khaya senegalensis*, Pharm. Biol. 39 (2001) 284–288. https://doi.org/10.1076/phbi.39.4.284.5921.

[40] T. Yuan, C.R. Zhang, S.P. Yang, J.M. Yue, Limonoids and triterpenoids from *Khaya senegalensis*, J. Nat. Prod. 73 (2010) 669–674. https://doi.org/10.1021/np1000158.

[41] M. Marzouk, S.M. Khalifa, A.H. Ahmed, A.M. Metwaly, H.S. Mohammed, H.A.A. Taie, LC/HR-ESI-MS/MS screening, phytochemical character-ization, and *in vitro* antioxidant and cytotoxic potential of *Jatropha integerrima* Jacq. extracts, Bioorg. Chem. 140 (2023) 106825.

[42] J.E. Robbers, M.K. Speedie, V.E. Tyler, Chapter 9: Alkaloids, Pharmacogn. Pharmacobiotechnology; Lippincott. Williams Wilkins Philadelphia, NY, USA (1996) 143–185.

[43] J.B. Harborne, Phytochemical Methods, Springer Netherlands, Dordrecht, 1980. https://doi.org/10.1007/978-94-009-5921-7.

[44] W.C. Evans, Trease and Evans' pharmacognosy, Elsevier Health Sciences, 1989.

[45] S. De, Y.N. Dey, A.K. Ghosh, Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphaphallus paeoniifolius* (Araceae), Int J Pharm Biol Res 1 (2010) 150–157.

[46] L.S. Melanchauski, A.P.G.S. Broto, T.M. Moraes, A.L.M. Nasser, A. Said, U.W. Hawas, K. Rashed, W. Vilegas, C.A. Hiruma-Lima, Gastroprotective and antisecretory effects of *Ailanthus excelsa* (Roxb), J. Nat. Med. 64 (2010) 109–113.

[47] T.J. Mabry, K.R. Markham, M.B. Thomas, Reag-

ents and Procedures for the Ultraviolet Spectral Analysis of Flavonoids BT-The Systematic Identi-fication of Flavonoids, in: T.J. Mabry, K.R. Mark-ham, M.B. Thomas (Eds.), Springer, Berlin, Heid-elberg, Berlin, Heidelberg, 1970: pp. 35–40. https://doi.org/10.1007/978-3-642-88458-0_4.

[48] H.A. Mohammed, R.A. Khan, A.A. Abdel-Hafez, M. Abdel-Aziz, E. Ahmed, S. Enany, S. Mahgoub, O. Al-Rugaie, M. Alsharidah, M.S.A. Aly, A.B.M. Mehany, M.M. Hegazy, Phytochemical Profiling, In Vitro and In Silico Anti-Microbial and Anti-Cancer Activity Evaluations and Staph GyraseB and h-TOP-IIβ Receptor-Docking Studies of Major Constituents of *Zygophyllum coccineum* L. Aque-ous-Ethanolic Extract and Its Subsequent Fra, Molecules 26 (2021) 577. https://doi.org/10.3390/molecules26030577.

[49] M.M. Hegazy, A.M. Metwaly, A.E. Mostafa, M.M. Radwan, A.B.M. Mehany, E. Ahmed, S. Enany, S. Magdeldin, W.M. Afifi, M.A. ElSohly, Biological and chemical evaluation of some African plants belonging to Kalanchoe species: Antitrypanosomal, cytotoxic, antitopoisomerase I activities and chem-ical profiling using ultra-performance liquid chro-matography/quadrupole-time-of-flight mass spect, Pharmacogn. Mag. 17 (2021) 6–15.

[50] S.A. Ahmed, H.A.A. Taie, W.A.A. Wahab, Anti-oxidant capacity and antitumor activity of the bio-active protein prepared from orange peel resid-ues as a by-product using fungal protease, Int. J. Biol. Macromol. 234 (2023) 123578.

[51] S.A.A. Saleh, A.A. Abd El-Galil, E.A.E. Sakr, H.A.A. Taie, F.A. Mostafa, Physiochemical, kinetic and thermodynamic studies on *Aspergillus wewitschiae* MN056175 inulinase with extraction of prebiotic and antioxidant Cynara scolymus leaves fructo-oligosaccharides, Int. J. Biol. Macro-mol. 163 (2020) 1026–1036.

[52] H. Tsugawa, T. Cajka, T. Kind, Y. Ma, B. Higgins, K. Ikeda, M. Kanazawa, J. Vander Gheynst, O. Fiehn, M. Arita, MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis, Nat. Methods 12 (2015) 523–526. https://doi.org/10.1038/nmeth.3393.

[53] S.P. Wong, L.P. Leong, J.H.W. Koh, Antioxidant activities of aqueous extracts of selected plants, Food Chem. 99 (2006) 775–783.

[54] M.O.W. Allah, A.A. Alrasheid, A.S. Elamin, Phy-tochemical screening, chemical composition and antioxidant activity of leaves and bark extracts from *Khaya senegalensis*, Adv. Biochem. 6 (2018) 32–38.

[55] L. Marius, T.T. Kadiatou, O. Salfo, C. Adjaratou, Antioxidants activities study of different parts of extracts of *Khaya senegalensis* (Desr.) A. Juss.(Meliaceae), Int J Pharmacol Res 10 (2020) e5522.

[56] F.N. Njayou, A.M. Amougou, R. Fouemene Tsayem, J. Njikam Manjia, S. Rudraiah, B. Bradley, J.E. Manautou, P. Fewou Moundipa, Antioxidant fractions of *Khaya grandifoliola* C. DC. and Entada africana Guill. et Perr. induce nuclear translocation of Nrf2 in HC-04 cells, Cell Stress Chaperones 20 (2015) 991–1000.

[57] L. Marius, F.Ã. Kini, D. Pierre, G.I. Pierre, *In vitro* antioxidant activity and phenolic contents of different fractions of ethanolic extract from *Khaya senegalensis* A. Juss.(Meliaceae) stem barks, African J. Pharm. Pharmacol. 10 (2016) 501–507.

[58] M. Ozaslan, I.D. Karagoz, I.H. Kilic, M.E. Guldur, Ehrlich ascites carcinoma, African J. Biotechnol. 10 (2011) 2375–2378.

[59] M. Jora, A.P. Burns, R.L. Ross, P.A. Lobue, R.Zhao, C.M. Palumbo, P.A. Beal, B. Addepalli, P.A. Limbach, Differentiating positional isomers of nucleoside modifications by higher-energy collisio-nal dissociation mass spectrometry (HCD MS), J. Am. Soc. Mass Spectrom. 29 (2018) 1745–1756.

[60] X. Chen, Y. Kang, S. Zeng, Analysis of stereoiso-mers of chiral drug by mass spectrometry, Chirality 30 (2018) 609–618.

[61] E. Bach, E.L. Schymanski, J. Rousu, Joint structur-al annotation of small molecules using liquid chro-matography retention order and tandem mass spec-trometry data, Nat. Mach. Intell. 4 (2022) 1224–1237.

[62] J. Hofmann, H.S. Hahm, P.H. Seeberger, K. Pagel, Identification of carbohydrate anomers using ion mobility–mass spectrometry, Nature 526 (2015) 241–244.

[63] Z.-H. Li, H. Guo, W.-B. Xu, J. Ge, X. Li, M. Alimu, D.-J. He, Rapid identification of flavonoid constituents directly from PTP1B inhibitive extract of raspberry (*Rubus idaeus* L.) leaves by HPLC–ESI–QTOF–MS-MS, J. Chromatogr. Sci. 54 (2016) 805–810.

[64] N. Fabre, I. Rustan, E. de Hoffmann, J. Quetin-Leclercq, Determination of flavone, flavonol, and flavanone aglycones by negative ion liquid chromatography electrospray ion trap mass spectrometry, J. Am. Soc. Mass Spectrom. 12 (2001) 707–715.

[65] A.M. El Sayed, S.M. Basam, E.-M. bellah A. El-Naggar, H.S. Marzouk, S. El-Hawary, LC–MS/MS and GC–MS profiling as well as the antimicrobial effect of leaves of selected Yucca species introduced to Egypt, Sci. Rep. 10 (2020) 17778.

[66] B. Alotaibi, W.A. Negm, E. Elekhnawy, T.A. El-Masry, M.E. Elharty, A. Saleh, D.H. Abdelkader, F.A. Mokhtar, Antibacterial activity of nano zinc

oxide green-synthesised from *Gardenia thailandica* triveng. Leaves against *Pseudomonas aeruginosa* clinical isolates: *in vitro* and *in vivo* study, Artif. Cells, Nanomedicine, Biotechnol. 50 (2022) 96–106.

[67] M.J. Alqahtani, E. Elekhnawy, W.A. Negm, S. Mahgoub, I.A. Hussein, Encephalartos villosus Lem. Displays a strong *in vivo* and *in vitro* antifungal potential against *Candida glabrata* clinical isolates, J. Fungi 8 (2022) 521.

[68] D.F. Felipe, L.Z.S. Brambilla, C. Porto, E.J. Pilau, D.A.G. Cortez, Phytochemical analysis of *Pfaffia glomerata* inflorescences by LC-ESI-MS/MS, Molecules 19 (2014) 15720–15734.

[69] M. Cortese, M.R. Gigliobianco, D.V. Peregrina, G. Sagratini, R. Censi, P. Di Martino, Quantification of phenolic compounds in different types of crafts beers, worts, starting and spent ingredients by liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1612 (2020) 460622.

[70] R. Jaiswal, J. Kiprotich, N. Kuhnert, Determination of the hydroxycinnamate profile of 12 members of the Asteraceae family, Phytochemistry 72 (2011) 781–790.

[71] H. Zhao, M. Zhu, K. Wang, E. Yang, J. Su, Q. Wang, N. Cheng, X. Xue, L. Wu, W. Cao, Identification and quantitation of bioactive components from honeycomb (*Nidus vespae*), Food Chem. 314 (2020) 126052.

[72] N. Nuengchamnong, K. Krittasilp, K. Ingkaninan, Characterisation of phenolic antioxidants in aqueous extract of *Orthosiphon grandiflorus* tea by LC–ESI-MS/MS coupled to DPPH assay, Food Chem. 127 (2011) 1287–1293.

[73] W. Ma, G. Yao, X. Huang, Q. Jia, J. Shen, Y. Chang, H. Ouyang, J. He, Comparison of the active components of *Aster tataricus* from different regions and related processed products by ultra-high performance liquid chromatography with tandem mass spectrometry, J. Sep. Sci. 43 (2020) 865–876.

[74] Y. Li, Y. Song, C. Liu, X. Huang, X. Zheng, N. Li, M. Xu, S. Mi, N. Wang, Simultaneous determina-tion of esculin and its metabolite esculetin in rat plasma by LC–ESI-MS/MS and its application in pharmacokinetic study, J. Chromatogr. B 907 (2012) 27–33.

[75] R. Marques, M.M. Sousa, M.C. Oliveira, M.J. Melo, Characterization of weld (*Reseda luteola* L.) and spurge flax (*Daphne gnidium* L.) by high-performance liquid chromatography–diode array detection–mass spectrometry in Arraiolos historical textiles, J. Chromatogr. A 1216 (2009) 1395–1402.

[76] M. Biswas, P.K. Haldar, A.K. Ghosh, Antioxidant and free-radical-scavenging effects of fruits of *Dre-gea volubilis*, J. Nat. Sci. Biol. Med. 1 (2010) 29.

[77] S. Quideau, D. Deffieux, C. Douat-Casassus, L. Pouységu, Plant polyphenols: chemical properties, biological activities, and synthesis, Angew. Chemie Int. Ed. 50 (2011) 586–621.

[78] H. Shabestarian, M. Homayouni-Tabrizi, M. Soltani, F. Namvar, S. Azizi, R. Mohamad, H. Shabestarian, Green synthesis of gold nanoparticles using sumac aqueous extract and their antioxidant activity, Mater. Res. 20 (2016) 264–270.

[79] N. Kumar, N. Goel, Phenolic acids: Natural versatile molecules with promising therapeutic applications, Biotechnol. Reports 24 (2019) e00370.

[80] H. Onohuean, A.I. Alagbonsi, I.M. Usman, K. Iceland Kasozi, A. Alexiou, R.H. Badr, G.E.-S. Batiha, J.O.C. Ezeonwumelu, Annona muricata linn and *Khaya grandifoliola* C. DC. Reduce oxidative stress *in vitro* and ameliorate plasmo-dium berghei-induced parasitemia and cytokines in BALB/c mice, J. Evidence-Based Integr. Med. 26 (2021) 2515690X211036669.

[81] A. Cavin, Investigation phytochimique de trois plantes indonésiennes aux propriétés antioxydantes et antiradicalaires: *Tinospora crispa* (Menisperma-ceae) Merremia emarginata (Convolvulaceae) et *Orophea enneandra* (Annonanceae)., (1999).

[82] S. V Jovanovic, S. Steenken, M. Tosic, B. Marjanovic, M.G. Simic, Flavonoids as antioxidan-ts, J. Am. Chem. Soc. 116 (1994) 4846–4851.

[83] H.-C. Lin, S.-H. Tsai, C.-S. Chen, Y.-C. Chang, C.-M. Lee, Z.-Y. Lai, C.-M. Lin, Structure–activity relationship of coumarin derivatives on xanthine oxidase-inhibiting and free radical-scavenging activities, Biochem. Pharmacol. 75 (2008) 1416–1425.

[84] A.J. Ruby, Antitumor and antioxidant activity of natural curcuminoids, Cancer Lett. 94 (1995) 783–799.

[85] M. Liu, J.C. Pelling, J. Ju, E. Chu, D.E. Brash, Antioxidant action via p53-mediated apoptosis, Cancer Res. 58 (1998) 1723–1729.