Egypt. J. Chem. Vol. **68**, No.2, pp. 243 -261 (2025)

Egyptian Journal of Chemistry

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

UPLC-QTOF-MS/MS Based Identification of Bioactive Compounds from *Garcinia livingstonei* Leaves and Evaluation of their Antioxidant and Antiarthritic Activities

Fatma H. Nossier[a]*, Azza R. Abdel Monem[a], Rehab M. S. Ashour[a], Marwa Mostafa Abdel-Aziz[b], Ahmed A. Al-Karmalawy[c], Ali M. Elsayed[a]

[a] *Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt*
[b] *Professor researcher of Medical Microbiology at The Regional Center for Mycology and Biotechnology, Al Azhar University, Cairo, Egypt*
[c] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Horus University- Egypt, New Damietta, 34518, Egypt. Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ahram Canadian University, 6th of October City, Giza 12566, Egypt.*

Abstract
Garcinia livingstonei T. Anderson (family Clusiaceae) is a rich source of different classes of bioactive compounds; mainly phenolics which possess beneficial pharmacological activities. In the present study the methanolic extract (ME) of the leaves was analyzed using UPLC-QTOF-MS/MS technique in both negative and positive modes. Its total phenolics and flavonoids content were determined using Folin-Ciocalteu and Aluminum chloride methods, respectively. Besides, the antioxidant activity of the ME and its different factions was estimated using three different assays (DPPH, FRAP, and ABTS), also the antiarthritic activity was evaluated using inhibition of protein denaturation, membrane stabilization and xanthine oxidase inhibitory % assays. Moreover, two different molecular docking studies were performed to investigate the potential antioxidant and antiarthritic activities of the identified compounds. UPLC-QTOF-MS/MS analysis revealed the presence of biflavonoids, benzophenones, flavonoids, xanthones, phenolic and organic acids. The total phenolics content was found to be 114.72 mg/g extract expressed as gallic acid equivalent, while the total flavonoids content recorded 53.1 mg/g extract expressed as rutin equivalent. Ethyl acetate fraction showed the most potent antioxidant and antiarthritic activities.

Keywords: *Garcinia livingstonei*, UPLC-QTOF-MS/MS analysis, antioxidant, antiarthritic, docking

1. Introduction

Herbal drugs had provided the humanity with bioactive compounds for many centuries, among them was *Garcinia livingstonei* T. Anderson, a member of Clusiaceae family. It includes about 250 species distributed in tropical and subtropical regions from Malaysia to Philippines **[1-3].** Genus *Garcinia* was reported to possess various pharmacological activities including antimicrobial, antioxidant, antiarthritic, skin lightening and anticancer activities **[4-7].** *Garcinia livingstonei* was traditionally used for treatment of diarrhea, cough and parasitic diseases **[8-9]**. *Garcinia* is a rich source of valuable phytoconstituents mainly phenolics such as biflavonoids (amentoflavone and morelloflavone), xanthones (alloathyriol and montixanthone) and benzophenones (guttiferone A, xanthochymol and 4,3′,4′-trihydroxy-2,6-dimethoxybenzophenone) **[10-15]**. Phenolics are aromatic compounds utilized as coloring and flavoring agents and antioxidants **[16, 17]**. The use of antioxidants to suppress the damaging effects of free radicals was of practical use in treatment of many diseases **[18]**. Different organs of many *Garcinia* species including *G. xanthochymus* roots and leaves, *G. merguensis* twigs and *G. morella* fruits were reported to possess antioxidant activity **[19-21]**. The antioxidant activity of stem wood, twigs and some isolated compounds of *G. livingstonei* were reported **[14]**. Also, the antioxidant activity of the methanolic extracts of *G. livingstonei* fruits and barks were documented **[22, 23]**. In addition, two isolated biflavonoids from the leaves of *G. livingstonei* were estimated for their antioxidant potential **[8]**. Little data were recorded about the total phenolics of *G. livingstonei* leaves, while most of the published data were concerned with its seeds and fruits **[24]**. Rheumatoid arthritis is an auto-immune disorder which causes joints inflammation. The use of

*Corresponding author e-mail: fatma_hassan197@yahoo.com; (Fatma H. Nossier)
Receive Date: 27 March 2024, Revise Date: 14 July 2024, Accept Date: 26 July 2024
DOI: 10.21608/ejchem.2024.279689.9518

synthetic drugs induces harmful side effects **[25, 26]** which encourages therapists to prescribe natural products as a safer choice for treatment of this disorder **[27].** Moreover, molecular docking is one of the most important tools in the long journey of drug discovery **[28].** It helps scientists to predict and/or investigate the proposed mechanism of action for a particular drug candidate. Besides, it saves time, effort, and money to introduce a new drug candidate for preclinical and/or clinical studies **[29].** The need to explore the phytochemical profile became mandatory to make use of plant health benefits. The present study provides identification of the bioactive compounds of *G. livingstonei* leaves to shed light on this interesting medically efficient natural remedy in correlation to its antioxidant and antiarthritic activities.

2. Experimental

2.1. Plant material

The leaves of *Garcinia livingstonei* were collected from AL Zohriya Garden during August 2021 and authenticated by Prof. Dr. Abd Haleem Abd El-Mogali, Chief researcher, Flora and Phyto Taxonomy Researches Department, Agriculture Museum, Giza, Egypt. Plant voucher sample were deposited at the pharmacy faculty's herbarium, Cairo University under the identification [no. 14.8.23]. One kilogram of air-dried leaves was grinded to fine powder then extracted by methanol (3 x 1 liter). The solvent was evaporated under reduced pressure to yield 300 g of dark brown sticky residue. The residue was successively fractionated with *n*-hexane, methylene chloride, ethyl acetate and *n*-butanol. The solvents were evaporated under reduced pressure to obtain 20, 12, 8 and 15 g of each fraction, respectively.

2.2. UPLC-QTOF-MS/MS analysis

The methanolic extract was analyzed using main column type XBridge C 18 (3.5 μm × 2.1 × 50 mm), temperature was adjusted at 40^{o} C with flow rate 0.3 mL/min. Mobile phase A was composed of 5 mM ammonium formate buffer (PH 8) containing 1% methanol. Mobile phase B was 100% acetonitrile. A linear gradient elution was programmed: 0 min., 90% A and 10% B; 21 min., 10% A and 90% B; 25.01 till 28 min., 90% A and 10% B. The method was described in details in supplementary material (S1).

2.3. Quantitative estimation of total phenolics

The total phenolics content of the methanolic extract of *G. livingstonei* was spectrophotometrically determined by Folin-Ciocalteu method and expressed as gallic acid equivalent **[30]**. The method was described in details in supplementary material (S2).

2.4. Quantitative estimation of total flavonoids content

Total flavonoids content of the methanolic extract of leaves of *G. livingstonei* was estimated by Aluminum chloride colorimetric method and expressed as rutin equivalent **[31]**. The method was mentioned in details in supplementary material (S3).

2.5. *In vitro* antioxidant activity

The methanolic extract of *G. livingstonei* and its fractions (*n*-hexane, methylene chloride, ethyl acetate and *n*-butanol) were tested for their antioxidant potential by three assays.

2.5.1. DPPH assay: was carried out according to the method of **Boly *et al.*, 2016** using Trolox as standard. The reduction in DPPH color intensity was measured at 540 nm **[32]**. The method was described in details in supplementary material (S4.1.).

2.5.2. FRAP assay: was performed following the method of **Benzie and Strain 1996** using Trolox as standard. The resulting blue color was measured at 593 nm **[33]**. The method was mentioned in details in supplementary material (S4.2.).
2.5.3. ABTS assay: was done obeying the method of **Arnao *et al.*, 2001.** The decrease in ABTS color intensity was measured at 734 nm **[34, 35]**. The method was described in details in supplementary material (S4.3.).

2.6. *In vitro* antiarthritic activity

The antiarthritic activity of *G. livingstonei* and its fractions (*n*-hexane, methylene chloride, ethyl acetate and *n*-butanol) was *in vitro* evaluated using three methods.

2.6.1. Inhibition of protein denaturation method: was estimated following the method of **Singh and Sharma 2016** with some modifications. Diclofenac sodium was used as standard and the reaction mixtures turbidity was measured at 660 nm **[36]**. The method was described in details in supplementary material (S5.1.).

2.6.2. Membrane stabilization method: was tested according to the modified method described by **Shinde *et al.*, 1999 [37]**. The method was mentioned in details in supplementary material (S5.2.).

2.6.3. Xanthine oxidase (XO) inhibitory method: was determined as previously reported **[38, 39]**. The method was described in details in supplementary material (S5.3.).
All determinations were carried out in triplicate and values were expressed as mean ± SD.

2.7. Molecular docking studies

All the identified compounds in the methanolic extract of *G. livingstonei* were subjected to two different molecular docking studies using the AutoDock Vina **[40]**, to investigate their potential antioxidant and antiarthritic activities. Visualization of results was performed using PyMOL software **[41]**. The two target receptors (Cytochrome C peroxidase and COX-2) were downloaded from the Protein Data Bank (PDB IDs: 2X08 and 1PXX, respectively), opened separately, cleaned from water molecules, 3D hydrogenate and energy minimized **[42]**. Each ligand was sketched in ChemDraw and prepared for docking by optimization of partial charges and energy minimization steps **[43]**. The most active compounds for each target receptor were selected for further studies.

3. Results & discussion

3.1. UPLC-QTOF-MS/MS analysis

The results of UPLC-QTOF-MS/MS analysis of the methanolic extract of *G. livingstonei* leaves in both negative and positive modes (Fig. 1 and 2 and Table 1) revealed the presence of different classes of phenolic compounds.

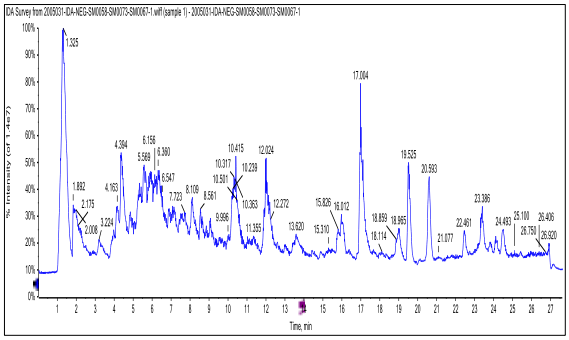


Fig. 1. Total UPLC-QTOF-MS/MS chromatogram of secondary metabolites in the methanolic extract of *Garcinia livingstonei* leaves (Negative mode)

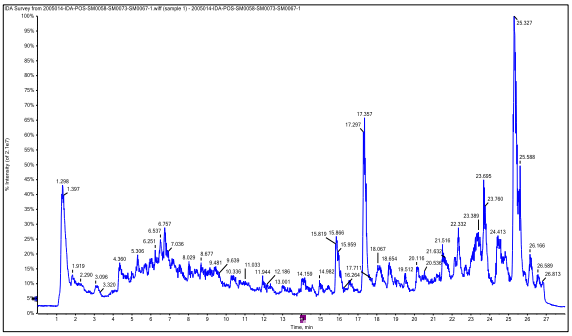


Fig. 2. Total UPLC-QTOF-MS/MS chromatogram of secondary metabolites in the methanolic extract of *Garcinia livingstonei* leaves (Positive mode)

Table 1. Metabolites identified in the methanolic extract of *Garcinia livingstonei* leaves using UPLC-QTOF-MS/MS technique

Compound No.	RT (min.)	Precursor ion *(m/z)*	Mode	Elemental Composition	Error	MS/MS *(m/z)*	Metabolite Assignment	Ref.
Phenolic and organic acids								
1.	**1.084**	**191.0191**	**$[M-H]^-$**	**$C_6H_7O_7^-$**	**2.5**	173.0088 111.0089	****Citric acid**	**[44]**
2.	**1.097**	**133.0138**	**$[M-H]^-$**	**$C_4H_5O_5^-$**	**4.9**	115.0033 89.0240	****Malic acid**	**[44]**
3.	**1.174**	**207.0152**	**$[M-H]^-$**	**$C_6H_7O_8^-$**	**-1.7**	189.0042 145.0450	****Hydroxy citric acid**	**[45]**
4.	**1.213**	**189.0048**	**$[M-H]^-$**	**$C_6H_5O_7^-$**	**9.6**	171.0436 127.0040	****Hydroxy citric acid lactone**	**[45]**
5.	**1.303**	**225.0605**	**$[M-H]^-$**	**$C_7H_{13}O_8^-$**	**-0.1**	207.0246 161.0451	*****Glucoheptonic acid**	**[47]**
6.	**1.370**	**359.0980**	**$[M-H]^-$**	**$C_{18}H_{15}O_8^-$**	**-6.2**	179.0569 161.0469	*****Rosmarinic acid**	**[51]**
7.	**1.446**	**315.0712**	**$[M-H]^-$**	**$C_{13}H_{15}O_9^-$**	**1.5**	153.0184 135.0306	*****Protocatechuic acid hexoside**	**[46]**
8.	**1.508**	**331.0670**	**$[M-H]^-$**	**$C_{13}H_{15}O_{10}^-$**	**3.1**	169.0150 125.0266	*****Gallic acid hexoside**	**[54]**
9.	**1.879**	**153.0197**	**$[M-H]^-$**	**$C_7H_5O_4^-$**	**9.2**	135.0081 109.0479	****Protocatechuic acid (Dihydroxybenzoic acid)**	**[46]**
10.	**2.227**	**169.0150**	**$[M-H]^-$**	**$C_7H_5O_5^-$**	**7.7**	151.0051 125.0249	****Gallic acid**	**[54]**
11.	**3.993**	**137.0237**	**$[M-H]^-$**	**$C_7H_5O_3^-$**	**2.6**	119.0156 93.0272	****Hydroxybenzoic acid**	**[55]**
Xanthones								
12.	**7.394**	**259.0238**	**$[M-H]^-$**	**$C_{13}H_7O_6^-$**	**0.3**	231.0293 187.0394	***Norathyriol (1,3,6,7-Tetrahydroxyxanthone)**	**[66]**
	9.235	**261.0387**	**$[M+H]^+$**	**$C_{13}H_9O_6^+$**	**2.4-**	243.0279 187.0377		

13.	**7.462**	**259.0234**	**[M-H]$^-$**	**$C_{13}H_7O_6^-$**	**1.1-**	231.0291 187.0398	****Bellidin (1,3,5,8-Tetrahydroxyxanthone)**	**[62]**
14.	**10.028**	**243.0297**	**[M-H]$^-$**	**$C_{13}H_7O_5^-$**	**3.6**	199.0401 143.0501	****Trihydroxyxanthone**	
15.	**11.392**	**273.0404**	**[M-H]$^-$**	**$C_{14}H_9O_6^-$**	**3.7**	258.0172 202.0270	***Alloathyriol (1,3,6-Trihydroxy-7-methoxyxanthone)**	**[62] [66]**
	7.093	**275.0534**	**[M+H]$^+$**	**$C_{14}H_{11}O_6^+$**	**-6**	260.0310 203.0342		
16.	**11.454**	**273.0428**	**[M-H]$^-$**	**$C_{14}H_9O_6^-$**	**-8.9**	258.0166 202.0242	****Trihydroxy methoxyxanthone**	
	7.081	**275.0544**	**[M+H]$^+$**	**$C_{14}H_{11}O_6^+$**	**-2.2**	260.0330 204.0430		
17.	**11.517**	**273.0407**	**[M-H]$^-$**	**$C_{14}H_9O_6^-$**	**4.9**	258.0167 202.0309	***Montixanthone (3,6,7-Trihydroxy-1-methoxyxanthen-9-one)**	**[62] [66]**
	7.195	**275.0530**	**[M+H]$^+$**	**$C_{14}H_{11}O_6^+$**	**-7.5**	260.0311 204.0426		
18.	**11.620**	**273.0399**	**[M-H]$^-$**	**$C_{14}H_9O_6^-$**	**1.9**	258.0162 202.0204	****Bellidifolin, bellidifodin (1,5,8-Trihydroxy-3 -methoxyxanthen-9-one)**	**[62] [66]**
	7.107	**275.0528**	**[M+H]$^+$**	**$C_{14}H_{11}O_6^+$**	**-8**	260.0298 204.0400		
19.	**12.747**	**257.0442**	**[M-H]$^-$**	**$C_{14}H_9O_5^-$**	**-0.9**	242.0224 186.0385	****Gentisin (Dihydroxy methoxyxanthone)**	**[62]**
20.	**13.854**	**329.1019**	**[M+H]$^+$**	**$C_{18}H_{17}O_6^+$**	**-0.2**	273.0402 231.0292	*****1,3,5,6-Tetrahydroxy-2 -(3-methylbut-2-enyl) xanthone**	**[69]**
21.	**13.895**	**227.0347**	**[M-H]$^-$**	**$C_{13}H_7O_4^-$**	**3.5**	199.0415 155.0505	****Dihydroxyxanthone**	
22.	**14.558**	**329.1010**	**[M+H]$^+$**	**$C_{18}H_{17}O_6^+$**	**-3**	273.0377 245.0420 217.0485	*****1,3,5,6-Tetrahydroxy-7-(3-methylbut-2-enyl) xanthone**	**[69]**
23.	**14.923**	**329.1025**	**[M+H]$^+$**	**$C_{18}H_{17}O_6^+$**	**-2.9**	273.0386 245.0440 217.0486	*****1,3,6,7-Tetrahydroxy-8-(3-methylbut-2-enyl) xanthone**	**[69]**
24.	**16.967**	**341.1015**	**[M-H]$^-$**	**$C_{19}H_{17}O_6^-$**	**-1.3**	326.0802 285.0397	****Dulxanthone A**	**[66]**
25.	**17.819**	**465.2307**	**[M+H]$^+$**	**$C_{28}H_{33}O_6^+$**	**7.5**	341.1061 285.0397	****Allanxanthone C**	**[69]**
Flavonoids								
26.	**5.207**	**461.1089**	**[M-H]$^-$**	**$C_{22}H_{21}O_{11}^-$**	**0.2-**	299.0568 284.0304	***** Diosmetin hexoside**	**[72]**
27.	**5.258**	**461.1077**	**[M-H]$^-$**	**$C_{22}H_{21}O_{11}^-$**	**0.2-**	299.0559 284.0321	***** Hispidulin hexoside**	**[73]**
28.	**5.456**	**593.1477**	**[M-H]$^-$**	**$C_{27}H_{29}O_{15}^-$**	**-4**	473.1046 447.0491 357.0616 327.0477	*****Orientin-2″-*O*-rhamnoside**	**[74]**
29.	**5.541**	**447.0905**	**[M-H]$^-$**	**$C_{21}H_{19}O_{11}^-$**	**-3.8**	357.0613 327.0507	****Orientin**	**[74]**
30.	**5.871**	**577.1529**	**[M-H]$^-$**	**$C_{27}H_{29}O_{14}^-$**	**-4.7**	457.1112 431.0860 311.0560	*****Isovitexin-2´´-*O*-rhamnoside**	**[74]**
31.	**5.897**	**645.1387**	**[M-H]$^-$**	**$C_{32}H_{35}O_{14}^-$**	**-2**	577.1527 457.1168 311.0541	*****Isovitexin-2´´-*O*-rhamnoside isoprenyl**	
32.	**6.096**	**447.0933**	**[M-H]$^-$**	**$C_{21}H_{19}O_{11}^-$**	**2.4**	357.0594 327.0495	*****Isoorientin**	**[74]**
	5.965	**449.1076**	**[M+H]$^+$**	**$C_{21}H_{21}O_{11}^+$**	**-0.4**	329.0662 299.0553		
33.	**6.195**	**431.0959**	**[M-H]$^-$**	**$C_{21}H_{19}O_{10}^-$**	**3.1-**	341.0659 311.0550	****Vitexin**	**[74] [76]**

34.	**6.430**	**431.0953**	**[M-H]$^-$**	**$C_{21}H_{19}O_{10}^-$**	**-4.7**	341.0651 311.0546	****Isovitexin**	**[72]** **[76]**
	7.084	**433.1106**	**[M+H]$^+$**	**$C_{21}H_{21}O_{10}^+$**	**-5.2**	313.0696 283.0593		
35.	**7.518**	**301.0705**	**[M+H]$^+$**	**$C_{16}H_{13}O_6^+$**	**-2.3**	286.0475 258.0527 153.0180	*****Hispidulin**	**[73]**
36.	**7.5493**	**301.0700**	**[M+H]$^+$**	**$C_{16}H_{13}O_6^+$**	**-2.2**	286.0468 258.0526 153.0179	*****Hispidulin isomer**	
37.	**7.746**	**301.0718**	**[M+H]$^+$**	**$C_{16}H_{13}O_6^+$**	**3.8**	286.0475 258.0519 153.0178	*****Diosmetin**	**[73]**
38.	**8.946**	**287.0550**	**[M-H]$^-$**	**$C_{15}H_{11}O_6^-$**	**0.1**	151.0042 135.0448	***Eriodictyol**	**[83]** **[84]**
	8.336	**289.0726**	**[M+H] $^+$**	**$C_{15}H_{13}O_6^+$**	**6.6**	259.0249 231.0281		
39.	**9.361**	**285.0398**	**[M-H]$^-$**	**$C_{15}H_9O_6^-$**	**1.7**	239.0423 151.0055	****Kaempferol**	**[55]** **[83]**
	9.749	**287.0546**	**[M+H]$^+$**	**$C_{15}H_{11}O_6^+$**	**-1.4**	241.0520 153.0168		
40.	**10.141**	**343.0816**	**[M-H]$^-$**	**$C_{18}H_{15}O_7^-$**	**1.2**	325.0646 313.0726 161.0578	*****5,7,4′-Tri-*O*-methyl quercetin**	**[85]**
41.	**10.476**	**271.0589**	**[M-H]$^-$**	**$C_{15}H_{11}O_5^-$**	**4.6-**	177.0178 151.0038 119.0489	****Naringenin**	**[55]**
42.	**10.671**	**269.0454**	**[M-H]$^-$**	**$C_{15}H_9O_5^-$**	**2.1**	225.0631 151.0131	****Apigenin**	**[55]** **[83]**
	11.003	**271.0594**	**[M+H]$^+$**	**$C_{15}H_{11}O_5^+$**	**-2.8**	153.0181 119.0508		
Tannins								
43.	**5.542**	**451.1217**	**[M-H]$^-$**	**$C_{21}H_{23}O_{11}^-$**	**-3.9**	289.0711 179.0633	*****Catechin-*O*-hexoside**	**[98]**
	6.336	**453.1390**	**[M+H]$^+$**	**$C_{21}H_{25}O_{11}^+$**	**-0.4**	291.0871 181.0502		
Biflavonoids								
Morelloflavone type (Flavan-(3→8″)-flavone)								
44.	**7.714**	**717.1430**	**[M-H]$^-$**	**$C_{36}H_{29}O_{16}^-$**	**-2.8**	565.0574 429.0574 403.0828	****Fukugiside (7″-*O*-Glucoside of morelloflavone)**	**[45]**
45.	**8.213**	**635.0466**	**[M-H]$^-$**	**$C_{30}H_{19}O_{14}S^-$**	**3.8-**	555.0926 429.0605	***Morelloflavone-7″-*O*-sulphate**	**[45]**
46.	**8.848**	**701.1467**	**[M-H]$^-$**	**$C_{36}H_{29}O_{15}^-$**	**-4.9**	539.0960 413.0645 125.0274	****Espicataside (Volkensiflavone-7″-*O*-glucoside)**	**[65]** **[45]**
	9.430	**703.1621**	**[M+H]+**	**$C_{36}H_{31}O_{15}^+$**	**-5.1**	541.1080 415.0775		
47.	**9.051**	**539.0952**	**[M-H]$^-$**	**$C_{30}H_{19}O_{10}^-$**	**-3.9**	413.0570 384.8872 151.0053	***Volkensiflavone**	**[66]** **[45]**
	10.410	**541.1129**	**[M+H]$^+$**	**$C_{30}H_{21}O_{10}^+$**	**-1**	415.0821 387.0869 153.0185		
48.	**9.966**	**555.0901**	**[M-H]$^-$**	**$C_{30}H_{19}O_{11}^-$**	**-3.8**	429.0605 403.0789 125.0248	***Morelloflavone**	**[101]**
49.	**10.688**	**555.0876**	**[M-H]$^-$**	**$C_{30}H_{19}O_{11}^-$**	**-8.3**	429.0608 403.0850 125.0267	****Fukugetin (Morelloflavone isomer)**	**[45]**
GB-1a type (flavanone-(3→8″)-flavanone)								
50.	**8.051**	**719.1551**	**[M-H]$^-$**	**$C_{36}H_{31}O_{16}^-$**	**-7.8**	593.1282 557.1069 431.0764 125.0237	****GB-2a-*O*-hexoside**	**[45]**

51.	**8.099**	**637.0507**	**[M-H]$^-$**	**$C_{30}H_{21}O_{14}S^-$**	**-4.3**	557.0959 431.0673 151.0040	***GB-2a-*O*-sulphate**	**[45]**
52.	**9.931**	**557.1028**	**[M-H]$^-$**	**$C_{30}H_{21}O_{11}^-$**	**-9.1**	431.0752 321.0365 295.0219 151.0028	**GB-2a**	**[45]**
53.	**11.061**	**541.1049**	**[M-H]$^-$**	**$C_{30}H_{21}O_{10}^-$**	**-4.9**	415.0816 151.0024 125.0258	**GB-1a**	**[102]**
Amentoflavone type (Flavone-(3→8″)-flavone)								
54.	**9.547**	**713.1466**	**[M-H]$^-$**	**$C_{37}H_{29}O_{15}^-$**	**-4.8**	551.0945 375.0510	***Hexoside of methyl amentoflavone**	
55.	**10.168**	**537.0800**	**[M-H]$^-$**	**$C_{30}H_{17}O_{10}^-$**	**-3**	375.0498	**Amentoflavone**	**[45]**
56.	**11.197**	**555.0931**	**[M+H]$^+$**	**$C_{30}H_{19}O_{11}^+$**	**1.6**	403.0548 377.0795	***3´´´-Hydroxyamentoflavone**	**[111]**
57.	**11.976**	**551.0939**	**[M-H]$^-$**	**$C_{31}H_{19}O_{10}^-$**	**-6.1**	399.0505 375.0492	***4´´´-Methyl amentoflavone**	**[111]**
IC3'–IIC3''' Linked Biflavonoids								
58.	**12.977**	**553.1109**	**[M-H]$^-$**	**$C_{31}H_{21}O_{10}^-$**	**-3.6**	401.1018 427.0814 125.0234	***Dihydro 3',3'''-binaringen methyl ether**	**[101]**
	14.457	**555.1251**	**[M+H]$^+$**	**$C_{31}H_{23}O_{10}^+$**	**4.3**	403.1152 153.0164		
Benzophenones								
59.	**2.921**	**263.0569**	**[M+H]$^+$**	**$C_{13}H_{11}O_6^+$**	**1.2-**	153.0183 137.0250	****Pentahydroxybenzophenone**	
60.	**2.998**	**263.0547**	**[M+H]$^+$**	**$C_{13}H_{11}O_6^+$**	**1.2-**	153.0159 137.0215	****Maclurin (Pentahydroxybenzophenone)**	**[114]**
61.	**8.150**	**247.0610**	**[M+H]$^+$**	**$C_{13}H_{11}O_5^+$**	**3.6**	153.0182 121.0280	****Tetrahydroxybenzophenone**	
62.	**19.098**	**601.3501**	**[M-H]$^-$**	**$C_{38}H_{49}O_6^-$**	**-3.8**	465.3467 329.2817 177.0209 109.0288	****Cambogin / 30-Epicambogin**	**[115]**
63.	**19.448**	**601.3505**	**[M-H]$^-$**	**$C_{38}H_{49}O_6^-$**	**3.5-**	465.3424 329.2730 177.0201 109.0298	****30-Epicambogin / Cambogin**	**[118]**
64.	**19.509**	**601.3479**	**[M-H]$^-$**	**$C_{38}H_{49}O_6^-$**	**-7.4**	449.1943 177.0196 109.0292	****Guttiferone k**	**[118]**
65.	**22.317**	**535.3046**	**[M+H]$^+$**	**$C_{33}H_{43}O_6^+$**	**-1.6**	479.2380 399.2495 343.1172 177.0183	****Propolone D hydroperoxide**	**[121]**
66.	**26.450**	**519.3096**	**[M+H]$^+$**	**$C_{33}H_{43}O_5^+$**	**-1.7**	463.2437 451.2594 177.0197	****Garcinielliptone I Hyperibone B Propolone C or 18-Epi-propolone C**	**[121]**

* Reported in *Garcinia livingstonei*
** Reported in genus *Garcinia*
*** First time reported in genus *Garcinia*

3.1.1. Phenolic and organic acids

Eleven phenolic and organic acids were detected in leaves of *G. livingstonei*. Compounds (1-5) were identified as citric acid, malic acid, hydroxy citric acid, hydroxy citric acid lactone and glucoheptonic acid. Their [M-H]$^-$ appeared at *m/z* 191.0191, 133.0138, 207.0152, 189.0042 and 225.0605, respectively. Their mass spectra showed characteristic product ions due to loss of H_2O [M-H-18]$^-$ and CO_2 [M-H-44]$^-$ groups **[44-47]**. Hydroxy citric acid, hydroxy citric acid lactone, malic acid, and citric acid were previously detected in *G. cowa*. Hydroxy citric acid plays an important role in the management of obesity while malic acid was used in skin preparations **[48-50]**.

Compound (6) was identified as rosmarinic acid with $[M-H]^-$ at *m/z* 359.0980 and product ions at *m/z* 179.0569 and 161.0469, that matched with the published data **[51]**. Rosmarinic acid was reported to possess anti-inflammatory and antioxidant effects **[52]**. Compounds (7-11) showed mass spectra of protocatechuic acid hexoside, gallic acid hexoside, protocatechuic acid, gallic acid and hydroxybenzoic acid; with $[M-H]^-$ at *m/z* 315.0712, 331.0670, 153.0197, 169.0150 and 137.0237, respectively. Their fragmentation patterns showed product ions due to loss of H_2O $[M-H-18]^-$ and CO_2 $[M-H-44]^-$ groups. Phenolic acid glycosides (compounds 7 and 8) showed product ions representing the loss of hexoside moiety $[M-H-162]^-$ **[53-55]**. Protocatechuic acid and hydroxybenzoic acid were previously detected in *G. mangostana* fruit, while gallic acid was detected in *G. indica* **[56, 57]**. Protocatechuic acid and gallic acid were reported to possess antioxidant and antibacterial activities. Hydroxybenzoic acid plays an important role as antidiabetic **[58-61]**.

3.1.2. Xanthones

Fourteen xanthones were identified in leaves of *G. livingstonei*. The spectra of xanthones revealed the presence of different substituents such as hydroxy, methoxy and isoprenyl groups, however it was difficult to confirm their position exactly. The fragmentation pattern of xanthones indicates the loss of H_2O $[M-H-18]^-$, CO $[M-H-28]^-$, CO_2 $[M-H-44]^-$, methyl $[M-H-15]^-$ and prenyl $[M-H-56]^-$ moieties as illustrated in supplementary data (Fig. S1). Four hydroxy xanthones isomers were represented in compounds (12, 13, 14 and 21) which were tentatively identified as norathyriol (1,3,6,7-tetrahydroxyxanthone) ($[M-H]^-$ at *m/z* 259.0238 and $[M+H]^+$ at *m/z* 261.0387), bellidin (1,3,5,8-tetrahydroxyxanthone) ($[M-H]^-$ at *m/z* 259.0234), trihydroxyxanthone ($[M-H]^-$ at *m/z* 243.0297) and dihydroxyxanthone ($[M-H]^-$ at *m/z* 227.0347); with their fragmentation patterns matched with the published data **[62]**. Norathyriol was isolated from *G. livingstonei* and was reported to possess EGFR-tyrosine kinase inhibitory and antibacterial activities **[63]**. Bellidin was isolated from *G. campestris* and exhibited anti-AChE activity **[64]**. Trihydroxyxanthone and dihydroxyxanthone were reported in *G. vieillardii* and *G. succifolia* Kurz with antimalarial activity **[14, 65**].

Compounds (15-19) represented five hydroxy methoxy xanthone isomers, which were tentatively identified as alloathyriol ($[M-H]^-$ at *m/z* 273.0404 and $[M+H]^+$ at *m/z* 275.0534), trihydroxy methoxy xanthone ($[M-H]^-$ at *m/z* 273.0428 and $[M+H]^+$ at *m/z* 275.0544), montixanthone ($[M-H]^-$ at *m/z* 273.0407 and $[M+H]^+$ at *m/z* 275.0530), bellidifodin ($[M-H]^-$ at *m/z* 273.0399 and $[M+H]^+$ at *m/z* 275.0528), and gentisin ($[M-H]^-$ at *m/z* 257.0442). Beside the above mentioned fragmentation pattern of xanthones, the mass spectra of these compounds showed product ions $[M-H-15]^-$ and/or $[M+H-15]^+$ due to loss of methyl group **[62, 66]**. Alloathyriol and montixanthone were isolated before from the stem wood of *G. livingstonei*, while bellidifolin and gentisin were isolated from *G. campestris* and *G. lutea*, respectively **[14, 64]**. Montixanthone was reported to possess antioxidant activity, while bellidifolin showed anti-AChE activity **[67, 68]**.

Three compounds (20, 22, and 23) were characterized as isoprenyl substituted hydroxy xanthone isomers with the same precursor ion at *m/z* 329 $[M+H]^+$, having daughter ion at *m/z* 273 $[M+H-56]^+$ due to loss of isoprenyl group. Their mass spectra were matched with the published data of 1,3,5,6-tetrahydroxy-2-(3-methylbut-2-enyl) xanthone, 1,3,5,6-tetrahydroxy-7-(3-methylbut-2-enyl) xanthone and 1,3,6,7-tetrahydroxy-8-(3-methylbut-2-enyl) xanthone **[69]**. Compounds (24 and 25) were corresponding to dulxanthone A ($[M-H]^-$ at *m/z* 341.1015) and allanxanthone C ($[M+H]^+$ at *m/z* 465.2307) **[66, 69]**. Dulxanthone A was isolated from *G. cowa* and exhibited antitumor activity, while allanxanthone C was isolated from *G. mackeaniana* and showed antimicrobial activity **[70, 71]**.

3.1.3. Flavonoids

Seventeen flavonoids were identified in the leaves of *G. livingstonei* varying between flavonoid aglycons, *O*-glycoside and *C*-glycosides.

O-Glycoside flavonoids

Two compounds (26 and 27) represent two isomers of flavonoid glycosides having the same parent ion ($[M-H]^-$, *m/z* 461.1089 and 461.1077) were distinguished as diosmetin hexoside and hispidulin hexoside, respectively. Daughter ions appeared at *m/z* 299 and 284 resulted from the loss of hexoside $[M-H-162]^-$ followed by loss of methyl $[M-H-162-15]^-$ moieties, respectively. The two isomers can be differentiated by peak ion at 256 *m/z* which was higher in diosmetin aglycone **[72, 73]**.

C-Glycoside flavonoids

Seven *C*-glycoside flavonoids (28-34) were detected in the leaves of *G. livingstonei* namely; orientin-2″-*O*-rhamnoside ($[M-H]^-$ at *m/z* 593.1477), orientin ($[M-H]^-$ at *m/z* 447.0905), isovitexin-2″-*O*-rhamnoside ($[M-H]^-$ at *m/z* 577.1529), isovitexin-2″-*O*-rhamnoside isoprenyl ($[M-H]^-$ at *m/z* 645.1387), isoorientin ($[M-H]^-$ at *m/z* 447.0933 and $[M-H]^+$ at *m/z* 449.1076), vitexin ($[M-H]^-$ at *m/z* 431.0959), and isovitexin ($[M-H]^-$ at *m/z* 431.0953 and $[M-H]^+$ at *m/z* 433.1106). The fragmentation patterns of these compounds showed fragmentation ions resulted from the inter-glycosidic cleavage of hexoside moiety $[M-H-120]^-$ and/or $[M+H-120]^+$ which is characteristic for *C*-attached hexoside which differ from the facile loss of the glucoside moiety (-162 amu) in case of *O*-attached hexoside. Isovitexin-2″-*O*-rhamnoside isoprenyl which showed product ion at *m/z* 577.1527 corresponding to $[M-H-68]^-$ due to loss of isoprenyl group **[74, 75]**. To differentiate between the isomers of *C*-glycoside flavonoids, mass spectra of isoorientin and isovitexin in positive mode showed peak ions $[(^{0,2}X)^+-CH_2O]^+$ (299 and 283 for isoorientin and isovitexin, respectively) more prominent than the peak ion $[(^{0,2}X)^+-CHO]^+$ (300 and 284 isoorientin and isovitexin, respectively). While in negative mode, isoorientin mass spectrum has product ion $[M-H-90]^-$ (357) higher than $[M-H-120]^-$ (327), while in isovitexin mass spectrum peak ion $[M-H-90]^-$ (341) exhibited higher intensity than that in vitexin **[74, 76]**. Vitexin and isovitexin were isolated from *G. hombroniana*, while orientin was isolated from *G. cowa*. *C*-Glycoside flavonoids were reported to possess antinociceptive, antioxidant, antiviral and antiglycation activities [**77-82**].

Flavonoid aglycones

Compounds (35-42) represent eight flavonoid aglycones, namely; hispidulin ($[M+H]^+$ at *m/z* 301.0705), hispidulin isomer ($[M+H]^+$ at *m/z* 301.0700), diosmetin ($[M+H]^+$ at *m/z* 301.0718 *m/z*), eriodictyol ($[M-H]^-$ at *m/z* 287.0550 and $[M+H]^+$ at *m/z* 289.0726), kaempferol ($[M-H]^-$ at *m/z* 285.0398 and $[M+H]^+$ at *m/z* 287.0546), 5,7,4´-tri-*O*-methyl quercetin ($[M-H]^-$ at *m/z* 343.0816), naringenin ($[M-H]^-$ at *m/z* 271.0589) and apigenin ($[M-H]^-$ at *m/z* 269.0454 and $[M+H]^+$ at *m/z* 271.0594) with characteristic fragmentation patterns (RDA fragmentation pathway) as described in supplementary data (Fig. S2), in addition to product ions due to loss of H_2O and CO moieties that matched with the previously published data **[55, 83, 84]**. To differentiate between hispidulin, hispidulin isomer and diosmetin, the product ion at 286 was the base peak in hispidulin while the peak ion at 258 *m/z* has higher intensity in diosmetin **[73]**. 5,7,4´-Tri-*O*-methyl quercetin mass spectrum showed in addition to RDA fragmentation pathway, peak ion $[M-H-30]^-$ at *m/z* 313.0726 due to loss of two methyl groups and product ion at *m/z* 161.0578 refers to $(^{1,3}B)^-$ of quercetin plus 14 amu (methyl group) **[85]**. Hispidulin was reported to exhibit anticancer, antioxidant and antiepileptic effects, while diosmetin plays an important role in treatment of colitis **[86-88]**. Eriodictyol was isolated from the seeds of *G. livingstonei* and exhibited antioxidant activity **[89, 90]**. Apigenin, naringenin, quercetin, and kaempferol were reported in other *Garcinia* species and were reported to exhibit anticancer, antioxidant and anti-inflammatory activities **[91-97]**.

3.1.4. Tannin

Compound (43) was identified as catechin-*O*-hexoside ($[M-H]^-$ at *m/z* 451.1217 and $[M-H]^+$ at *m/z* 453.1390) with fragment ion due to loss of hexoside moiety to give the characteristic peak of catechin ($[M-H-162]^-$ at *m/z* 289.0711 and $[M+H-162]^+$ at *m/z* 291.0871) **[98]**. Catechin was effective as antidiabetic, anticancer, and antiarthritic **[99]**.

3.1.5. Biflavonoids

Biflavonoids are flavonoid dimers result from the connection of two units of flavone, flavanone, flavanonol, flavonol or aurones or mixtures of them, in addition to chalcone and isoflavone dimers **[100]**. The fragmentation pattern of biflavonoids includes cleavage at positions 1/3 and 0/4 in the flavanone part more than in flavone part, producing characteristic product ions, $(^{1,3}IB)^-$ and $(^{1,3}IIB)^-$, in addition to product ions due to loss of H_2O $[(^{1,3}IIB) - H_2O]^-$ and $[(^{1,3}IB) - H_2O]^-$ (Fig. S3). Fifteen biflavonoids were identified from the different types of biflavonoids depending on position and type of the linkage between the two flavonoids. Another characteristic peak ions were also detected at *m/z* 151 and 125 corresponding to A ring fragments $(^{1,3}IA)^-$ or $(^{1,3}IIA)^-$, and $(^{1,4}IA)^-$ or $(^{1,4}IIA)^-$ ions, respectively **[101]**.

Biflavonoids of morelloflavone type

The fragmentation pattern of this type of biflavonoids shows the loss of $C_6H_6O_3$ group (126 amu) followed by loss of CO moiety (28 amu) as described in supplementary data (Fig. S4). Six compounds (44-49) of morelloflavone type biflavonoids were identified in leaves of *G. livingstonei* namely; fukugiside ($[M-H]^-$ at *m/z* 717.1430), morelloflavone-7″-*O*-sulphate ($[M-H]^-$ at *m/z* 635.0466), espicataside ($[M-H]^-$ at *m/z* 701.1467 and $[M-H]^+$ at *m/z* 703.1621), volkensiflavone ($[M-H]^-$ at *m/z* 539.0952 and $[M-H]^+$ at *m/z* 541.1129), morelloflavone ($[M-H]^-$ at *m/z* 555.0901) and its isomer fukugetin ($[M-H]^-$ at *m/z* 555.0876) **[45, 66, 102]**. All these compounds showed characteristic product ion $[M-H-126]^-$ resulted from the loss of $C_6H_6O_3$ group which undergoes further loss of CO group, in addition to $(^{1,4}IA)^-$, $(^{1,4}IIA)^-$, $(^{1,3}IA)^-$ and $(^{1,3}IIA)^-$ ions **[101]**. Espicataside showed additional peak ion $[M-H-162]^-$ at *m/z* 539.0960 due to loss of hexose moiety, while morelloflavone-7″-*O*-sulphate showed peak ion $[M-H-80]^-$ at *m/z* 555.0926 due to loss of SO_3 group (Fig. S4). Fukugiside has characteristic fragmentation pathway leading to the loss of $C_7H_4O_4$ $[M-H-152]^-$ to give peak ion at *m/z* 565 followed by the loss of hexoside moiety to yield the product ion at 403 *m/z* **[45]**. Volkensiflavone, morelloflavone, and morelloflavone-7″-*O*-sulphate were isolated from *G. livingstonei* and were reported to have skin lightening and antioxidant activities **[7, 103]**. Espicataside was isolated from *G. madruno*, while fukugetin and fukugiside were isolated from *G. brasiliensis* and they were reported to have antioxidant and cytotoxic activities **[89, 104-106]**.

Biflavonoids of GB-1a type

The fragmentation pattern of this type of biflavonoids showed the loss of $C_6H_6O_3$ group (126 amu), followed by loss of $C_6H_6O_2$ (110 amu) or loss of $C_8H_8O_2$ (136 amu) as illustrated in supplementary data (Fig. S5). Four biflavonoids of GB-1a type (compounds 50-53) were identified in the negative mode namely; GB-2a hexoside, GB-2a-*O*-sulphate, GB-2a, and GB-1a; with parent ions $[M-H]^-$ at *m/z* 719.1551, 637.0507, 557.1028 and 541.1049, respectively. These compounds had product ion $[M-H-126]^-$ due to loss of $C_6H_6O_3$ group followed by loss of 136 amu ($C_8H_8O_2$ group). GB-2a-*O*-sulphate and GB-2a-*O*-hexoside showed additional product ions at *m/z* 557.0959 $[M-H-80]^-$ and 557.1069 $[M-H-162]^-$ due to loss of SO_3 and hexoside moieties, respectively. The fragmentation pattern of GB-2a (naringenin-3-8″-eriodictyol) was similar to GB-1 (naringenin-3→8″-dihydrokaempferol), but they can be differentiated by fragment ion at *m/z* 321.0365, present only in the mass spectrum of GB-2a, that resulted from the loss of B ring of eriodictyol $[(M-H-126)-110]^-$ **[101, 102]**. GB-1a, GB-2a, and GB-2a-*O*-hexoside were isolated from *Garcinia* species and were reported to have antiplasmodial, anti-inflammatory, and monoamine oxidase inhibitory activities **[104, 107-110]**.

Biflavonoids of amentoflavone type

Fragmentation pattern of this type of biflavonoid includes the cleavage of the C ring of flavonoid part II at position 0/4 and loss of $C_9H_6O_3$ (-162 amu) as presented in supplementary data (Fig. S6) **[45]**. Four compounds (54-57) of amentoflavone type biflavonoids were identified in leaves of *G. livingstonei* namely; methyl amentoflavone hexoside ($[M-H]^-$ at *m/z* 713.1466), amentoflavone ($[M-H]^-$ at *m/z* 537.0800), 3´´´-hydroxyamentoflavone ($[M-H]^+$ at *m/z* 555.0931), and 4´´´-methyl amentoflavone ($[M-H]^-$ at *m/z* 551.0939). Methyl amentoflavone was identified by the product ion at 551 which was higher than amentoflavone by 14 amu and the characteristic fragment ion of amentoflavone at *m/z* 375.0492, indicating that methyl group was at 4´´´ position.

Also, methyl amentoflavone hexoside was identified by the presence of 162 amu (hexoside moiety) higher than 551 **[45, 102, 111]**. Amentoflavone and methoxyamentoflavone were previously isolated from *G. livingstonei* and the twigs of *G. xanthochymus* and showed antioxidant and antiviral activities **[8, 20, 112]**.

IC3'-IIC3''' Linked biflavonoids type

Compound (58) showed precursor ions $[M-H]^-$ at *m/z* 553.1109 and $[M+H]^+$ at *m/z* 555.1251, less than that of 3',3'''-binaringen methyl ether by (2 amu) suggesting the presence of dihydro-3',3'''-binaringen methyl ether. This assignment with confirmed by comparing the product ions at *m/z* 427.0814 $(^{0,4}IIB\text{-}H_2O)^-$ and 401.1018 $(^{1,3}IIB)^-$ with that of 3',3'''-binaringen methyl ether (*m/z* 429 and 403) **[101]**.

3.1.6. Benzophenones

Benzophenones are natural compounds that have two benzene rings attached *via* carbonyl group. Eight benzophenones were identified in the chromatogram of *G. livingstonei*. Compounds (59 and 60) showed $[M+H]^+$ at *m/z* 263.0569 and 263.0547, respectively, the fragmentation pattern of these compounds showed peak ion at 153 *m/z* due to loss of benzene ring with one hydroxyl group $[M+H-77-17]^+$. Compound (61) showed $[M+H]^+$ at *m/z* 247.0610 with product ion at 153 *m/z* due to loss of benzene ring with two hydroxyl groups $[M+H-77-17-16]^+$. The fragmentations of these three compounds were in accordance with pentahydroxy (compound 59 and 60) and tetrahydroxy (61) benzophenones, containing three hydroxyl groups in the other benzene ring. The distribution of hydroxyl groups however, can't be determined by LC/MS only **[113, 114]**. Tetra and penta hydroxy benzophenones were reported in *Garcinia* species (*G. cantleyana* and *G. mangostana*) and were reported to have anti-cancer effect **[91, 115-117]**.

Two isomers cambogin (compound 62) and 30-epicambogin (compound 63), with parent ion $[M-H]^-$ at *m/z* 601.3501 and 601.3505, respectively, showed characteristic product ions at *m/z* 177 and 109. Cambogin and its isomer 30-epicambogin gave product ion at *m/z* 465 indicating the facile loss of geranyl unit (136 amu) followed by loss of prenyl unit then C_6H_8 unit from the pyran ring as illustrated in supplementary data (Fig. S7) to give product ion at 329. Guttiferone K (compound 64) with precursor ion $[M-H]^-$ at *m/z* 601.3479 and the characteristic fragments 177.0196 and 109.0292 *m/z* was matched with the published data **[118**]. Cambogin and 30-epicambogin were isolated from *G. multiflora* and *G. indica*, respectively, while guttiferone K was reported in *G. yunnanensis*, these compounds exhibited anticancer activity [**66, 119, 120]**.

Compound (65) with precursor ion $[M+H]^+$ at *m/z* 535.3046, suggested the presence of oxygenated propolone D (519 *m/z* + 16). This suggestion was supported by the presence of product ion at 399.2495 *m/z* $[M+H-136]^+$ due to loss of geranyl group resembling the peak ion at 383 *m/z* in propolone D $[M+H-136]^+$, so this compound was tentatively identified as propolone D hydroperoxide **[121]**.

Mass spectrum of compound (66) with $[M+H]^+$ at *m/z* 519.3096 and product ions at 463.2437 *m/z* $[M+H-56]^+$ and 451.2594 *m/z* $[M+H-68]^+$ due to loss of prenyl and isoprenyl groups, respectively. This fragmentation represented garcinielliptone I benzophenone or its isomers namely, hyperibone B, propolone C or 18-epi-propolone C, that was difficult to distinguish between them **[121, 122]**. Garcinielliptone I was reported to possess anti-inflammatory activity **[123]**.

3.2. Total phenolics and total flavonoids content

The total phenolics content of the methanolic extract of *G. livingstonei* leaves expressed as gallic acid equivalent was found to be 114.72 mg GAE/g extract. Calibration curve of standard gallic acid was established (R^2= 0.9961) (Y= 0.0031x - 0.0564) by taking the average of the readings of 6 replicates (Fig. S8). While, the total flavonoids content expressed as rutin equivalent was found to be 53.1 mg RE/g extract. Standard rutin calibration curve was established (R^2= 0.9981) (Y= 0.0032x - 0.0398) by taking the average of the readings of 6 replicates (Fig. S9). This is the first determination of the total phenolics in the leaves of *G. livingstonei.* Previously published data reported the total phenolics content in other plant parts rather than the leaves. The total phenolics content of the fruits was 115.5 mg GAE/g [22]. In another study, the total phenolics content in the different fruit parts and seeds was determined, it was found that the epicarp had the highest total phenolics (174.02 ± 0.17 mg GAE/g), while the highest flavonoids content was found in the seeds (99.98 ± 0.23 μg QE/g) **[24]**.

3.3. *In vitro* antioxidant activity

The antioxidant activity of the methanolic extract of leaves of *G. livingstonei* and its fractions (*n*-hexane, methylene chloride, ethyl acetate and *n*-butanol) was evaluated using DPPH, FRAP and ABTS assays compared to trolox as standard.

3.3.1. DPPH assay

The percentage of inhibition on the stable radical DPPH due to the electron donating character of the tested samples was measured (Fig. S10) **[124]**. IC_{50} was then determined (Fig. 3), the ethyl acetate fraction of *G. livingstonei* was the most potent fraction with IC_{50} = 13.7 μg/mL compared to the standard trolox which exhibited IC_{50} =24.24 μg/mL followed by methylene chloride and *n*-hexane fractions with IC_{50} = 23.11 and 23.33 μg/mL, respectively. The *n*-butanol fraction showed the same DPPH activity as the standard (IC_{50} =24.27 μg/mL). Meanwhile, the methanolic extract was the least effective one with the highest IC_{50} value (43.68 μg/mL).

3.3.2. FRAP assay

The FRAP assay measures the reduction in ferric ion (Fe^{+3}) to ferrous ion (Fe^{+2}) due to the presence of electrons donor **[125]**. The results were expressed as μM trolox equivalent/mg sample using linear dose-inhibition curve of trolox (R^2= 0.9993) (Y= 0.0014x - 0.0603) (Fig. S11). The *n*-butanol fraction followed by the ethyl acetate fraction were the most potent (1680.23 and 1611.19 μM trolox equivalent/mg, respectively). *n*-Hexane and methylene chloride fractions have nearly the same values (861.78

and 811.19 μM trolox equivalent/mg, respectively), which were more effective than the methanolic extract (621.52 μM trolox equivalent/mg) (Fig. 4).

3.3.3. ABTS assay

This assay measures the ability of the tested sample to scavenge the ABTS cation radicals **[126]**. The results were expressed as μM trolox equivalent/mg sample using linear dose-inhibition curve of Trolox. (R^2= 0.9948) (Y= 0.1089x - 3.5686) (Fig. S12). The results showed that the ethyl acetate fraction was the most potent (2997.80 μM trolox equivalent/mg), followed by *n*-butanol fraction (2060.93 μM trolox equivalent/mg). Methylene chloride and *n*-hexane fractions have nearly the same potency (1725.42 and 1617.85 μM trolox equivalent/mg, respectively). The least effective sample was the methanolic extract (1508.21 μM trolox equivalent/mg) (Fig. 5).

As shown from the previous results, the ethyl acetate and *n*-butanol fractions were the most potent, while the methanolic extract was the least potent in the three assays. This may be due to the presence of higher concentration of phenolic compounds like flavonoids and biflavonoids in the ethyl acetate and *n*-butanol fractions **[8, 126]**.

The present study is the first evaluation of the antioxidant activity of the methanolic extract and its different fractions of the leaves of *G. livingstonei*. Previous trials on the other parts of the plant showed that the methanol and combined ethyl acetate and acetone extracts of stem wood recorded IC_{50} =30.3 and 53.6 μg/mL, respectively, in DPPH assay. While, the aqueous and methanol extracts of the bark showed IC_{50} = 0.35 and 0.39 μg/mL, respectively **[23]**. The methanolic extract of fruits from Florida showed IC_{50} = 108.4 μg/mL **[22]**. Also, the methanol extract of twigs was assessed using DPPH assay (IC_{50} = 100.1 μg/mL) **[14]**. The isolated compounds were also tested for their antioxidant activity, amentoflavone and 4′-monomethoxyamentoflavone, isolated from *G. livingstonei* of South Africa, recorded 0.9 and 2.2 Trolox equivalent antioxidant capacity, respectively **[8]**. 3′,4,4′,6-Tetrahydroxy-2-methoxybenzophenone was the most active antioxidant compared to vitamin C **[14]**.

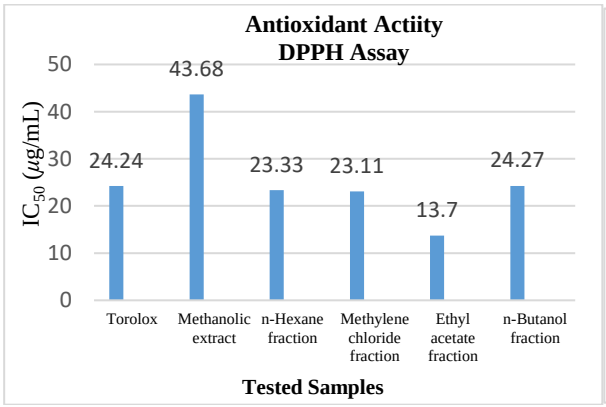


Fig. 3. IC_{50} values of the methanolic extract and its fractions from *Garcinia livingstonei* in the DPPH assay

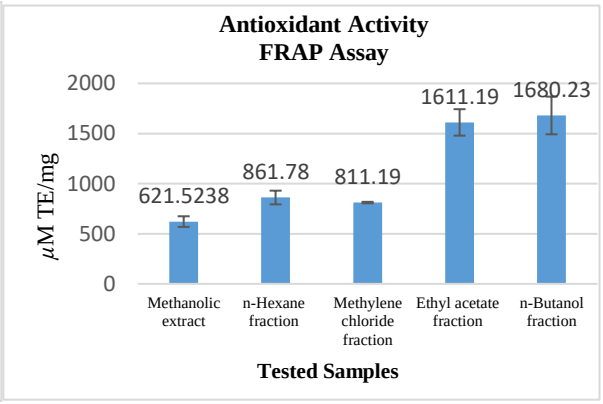


Fig. 4. Antioxidant activity of methanolic extract of *Garcinia livingstonei* and its fractions using FRAP assay

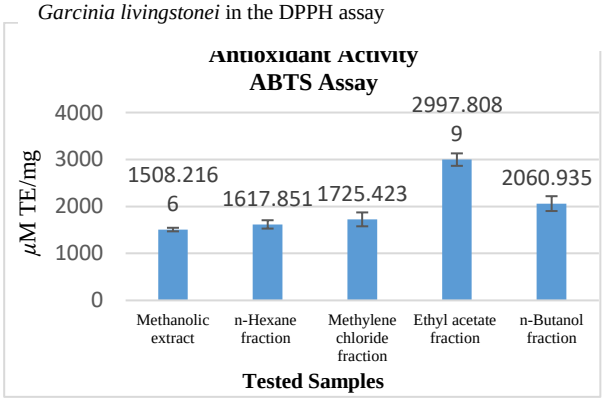


Fig. 5. Antioxidant activity of methanolic extract of *Garcinia livingstonei* and its fractions using ABTS assay

3.4. *In vitro* antiarthritic activity

The antiarthritic activity of the methanolic extract of leaves of *G. livingstonei* and its fractions (*n*-hexane, methylene chloride, ethyl acetate and *n*-butanol) was evaluated *in vitro* using inhibition of protein denaturation, membrane stabilization and xanthine oxidase inhibitory methods (Fig. S13-S15). The antiarthritic efficacy in these three methods is exhibited by preventing the denaturation of albumin which leads to release of antigens causing hypersensitivity reaction, or by decreasing the release of the inflammatory harmful mediators from lysosomes and moreover by inhibition of xanthine oxidase enzyme which is responsible for the formation of xanthine from hypoxanthine and consequently the formation of uric acid that lads to exposure to gouty

arthritic, so inhibition of this enzyme aid in the treatment of gouty arthritis **[127-130]**. The results revealed that the ethyl acetate fraction followed by methylene chloride possess the most potent antiarthritic activity with the lowest IC_{50} in the three different assays compared to positive controls (diclofenac sodium, indomethacin and allopurinol, respectively) (Fig. 6-8). The activity of ethyl acetate fraction as antiarthritic may be attributed to the presence of flavonoids, bioflavonoids and other phenolic compounds. These results support the relation between the antioxidants and rheumatoid arthritis as mentioned in previous publications as the increased free radical resulting from xanthine oxidase or oxidative stress are of major causes of rheumatoid **[131, 132]**. *Garcinia* was reported to possess antiarthritic activity exemplified by fruits of *G. cambogia* **[15]** and the garcinol enriched fraction isolated from *G. indica* fruits **[133]**. This is the first report on the antiarthritic activity of *G. livingstonei* using these three assays.

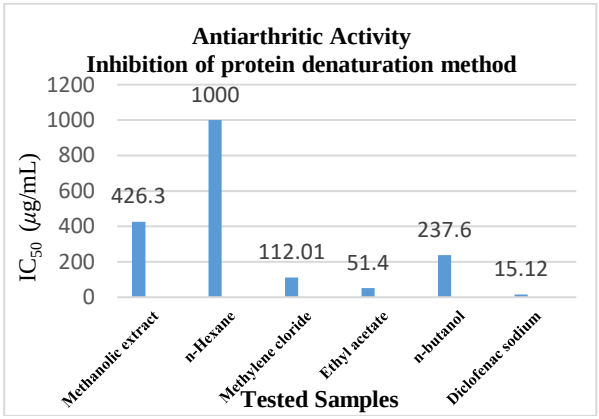


Fig. 6. IC_{50} of the methanolic extract and its fractions from *Garcinia livingstonei* compared to diclofenac sodium

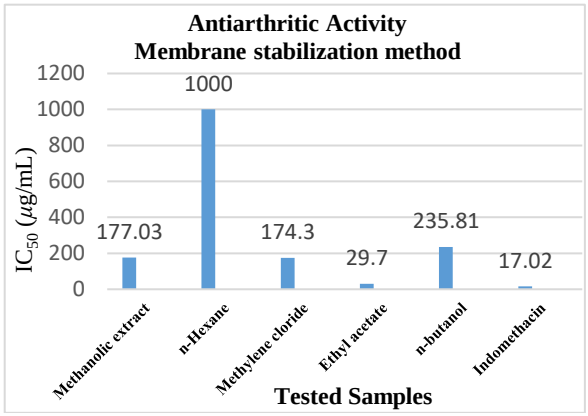


Fig. 7. IC_{50} of the methanolic extract and its fractions from *Garcinia livingstonei* compared to indomethacin

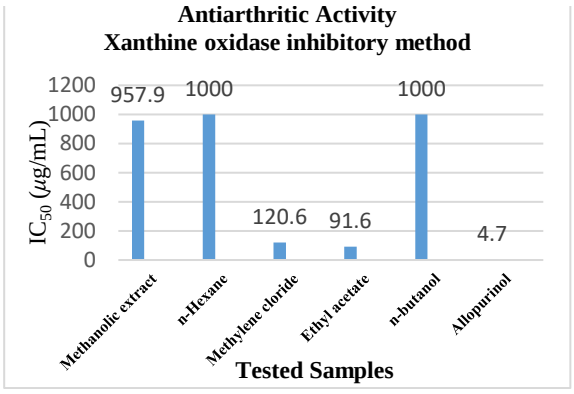


Fig. 8. IC_{50} of the methanolic extract and its fractions from *Garcinia livingstonei* compared to allopurinol

3.5. Molecular docking studies

First, visualizing the binding pocket of each target receptor was done to identify the co-crystallized ligand and its binding mode as well. For the cytochrome C peroxidase receptor; Arg184 and Val45 were the most crucial amino acids to induce the antagonistic effect; however, for COX-2; Ser530 and Tyr385 were the most important ones. Isovitexin-2"-*O*-rhamnoside isoprenyl, hexoside of methyl amentoflavone, and dihydro-3',3'''-binaringen methyl ether candidates were found to be the most promising against cytochrome C peroxidase target. Their binding scores were recorded to be -10-24, -9.45, and -9.25 kcal/mol, respectively, which were greatly higher than that of the docked co-crystallized ascorbic acid (-5.62 kcal/mol). Table 2 clarifies that isovitexin-2"-*O*-rhamnoside isoprenyl formed seven hydrogen bonds with Arg184, His181, Ser185, Lys179 (2), Leu177, and pro44. Besides, hexoside of methyl amentoflavone showed one hydrogen bond with Arg184 and one pi-cation interaction with Lys183. Moreover, dihydro-3',3'''-binaringen methyl ether achieved one pi-cation interaction with Arg184, two hydrogen bonds with Lys183 and Ala83 and two pi-hydrogen interactions with His181 and Ala36. These interactions suggested a strong binding affinity and stable interactions with the target proteins, indicating potential antioxidant activity.

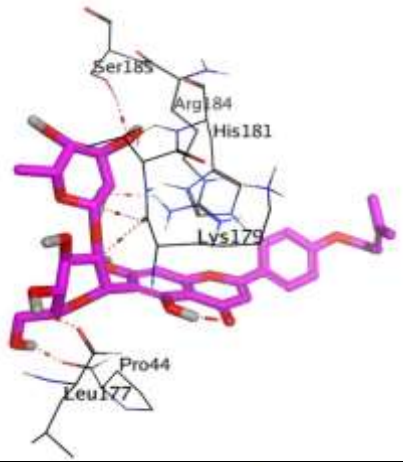
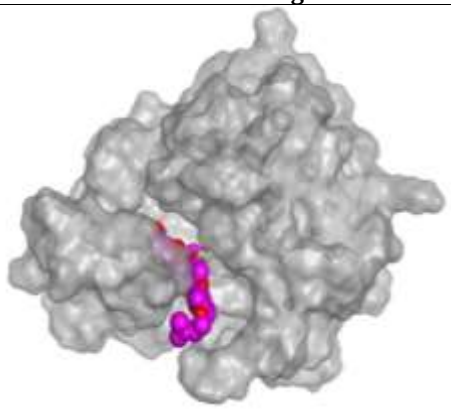
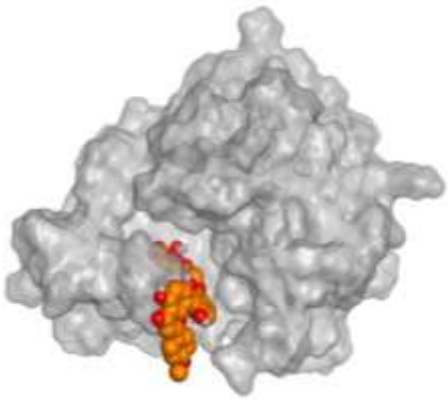
On the other hand, protocatechuic acid hexoside, 1,3,5,6-tetrahydroxy-7-(3-methylbut-2-enyl) xanthone, and 5,7,4´-tri-*O*-methyl quercetin were found to be the most promising candidates against COX-2 target with binding scores of -7.14, -7.01, and -6.86 kcal/mol, respectively. These binding scores were even superior to that of the docked co-crystallized diclofenac which achieved a binding score of -6.71 kcal/mol. According to these results we can suggest a potential antiarthritic activity from the prementioned compounds.

Table 3 shows that protocatechuic acid hexoside formed three hydrogen bonds with Tyr385, Met522, and Val523. However, 1,3,5,6-tetrahydroxy-7-(3-methylbut-2-enyl) xanthone bound Ser530 with a hydrogen bond and 5,7,4´-tri-*O*-methyl quercetin showed the formation of a hydrogen bond with Met522.

Accordingly, the previously discussed binding scores and similar binding modes of the most promising compounds from the methanolic extract of *Garcinia livingstonei* proposed their potential antioxidant and antiarthritic activities as well. The previous results revealed that the most potential compounds belong to flavonoid, bioflavonoids, and xanthones those were reported for their antioxidant and antiarthritic activities. By analyzing the molecular docking result, we can infer that the compounds with strong binding affinities and stable interactions (e.g., isovitexin-2"-*O*-rhamnoside isoprenyl, hexoside of methyl amentoflavone, dihydro-3',3'"-binaringen methyl ether) contribute significantly to the antioxidant activity, in addition protocatechuic acid hexoside, 1,3,5,6-tetrahydroxy-7-(3-methylbut-2-enyl) xanthone, and 5,7,4´-tri-*O*-methyl quercetin contribute significantly to the observed *in vitro* antiarthritic activity and resulted in that the ethyl acetate was the most active fraction almost in all assays. This correlation provides mechanistic insights into how specific compounds within the plant extract exert their biological effects, highlighting their potential therapeutic applications as antioxidant and antiarthritic.

Therefore, we recommend further preclinical and clinical studies especially for compounds (isovitexin-2"-*O*- rhamnoside isoprenyl, hexoside of methyl amentoflavone, and dihydro-3',3'"-binaringen methyl ether) and compounds (protocatechuic acid hexoside, 1,3,5,6-tetrahydroxy-7-(3-methylbut-2-enyl) xanthone, and 5,7,4´-tri-*O*-methyl quercetin) as potential antioxidant and antiarthritic candidates, respectively.

Table 2. 3D binding interactions and positioning of compounds (isovitexin-2´´-*O*-rhamnoside isoprenyl, hexoside of methyl amentoflavone, and dihydro-3',3'"-binaringen methyl ether) within the binding pocket of cytochrome C peroxidase (PDB ID: 2X08) target receptor

Compound	3D Interactions	3D Positioning
Isovitexin-2"-*O*-rhamnoside isoprenyl		
Hexoside of methyl amentoflavone		

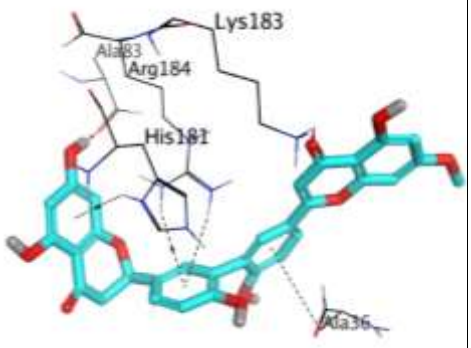
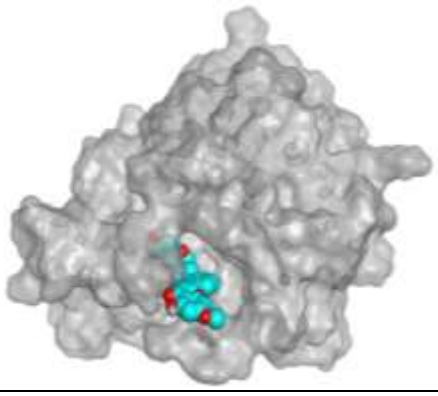
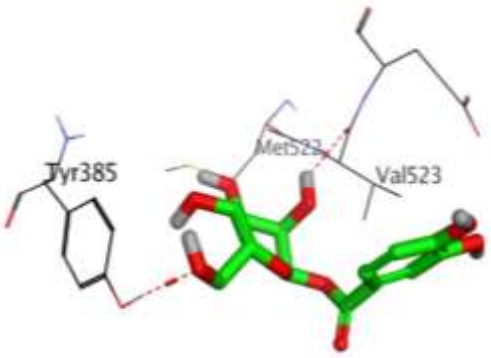
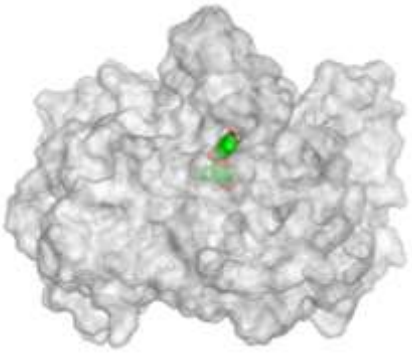
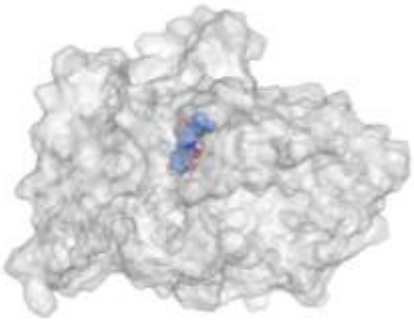
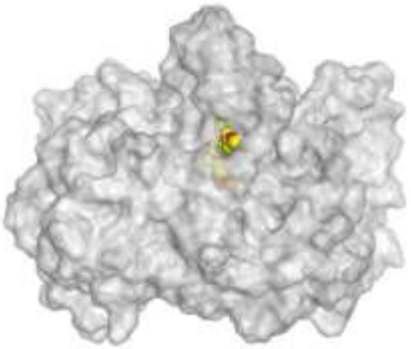
Dihydro-3',3'''-binaringen methyl ether		

Table 3. 3D binding interactions and positioning of compounds (protocatechuic acid hexoside, 1,3,5,6-tetrahydroxy-7-(3-methylbut-2-enyl) xanthone, and 5,7,4´-tri-*O*-methyl quercetin) within the binding pocket of COX-2 (PDB ID: 1PXX) target receptor

Compound	3D Interactions	3D Positioning
Protocatechuic acid hexoside		
1,3,5,6-Tetrahydroxy-7-(3-methylbut-2-enyl) xanthone		
5,7,4´-Tri-*O*-methyl quercetin		

4. Conclusion

Sixty-six compounds, belonging to biflavonoids, xanthones, tannins, benzophenones, flavonoids, phenolic and organic acids, were identified in *G. livingstonei* leaves for the first time by UPLC-QTOF-MS/MS technique. Among them rosmarinic acid, hexosides of protocatechuic and gallic acids, and hydroxy citric acid were detected here for the first time in *G. livingstonei*. *G. livingstonei* leaves proved its effectiveness as antioxidant, especially the phenolics rich ethyl acetate and butanol fractions. Also, the ethyl acetate fraction was the most effective as antiarthritic. Furthermore, molecular docking studies recommend compounds (isovitexin-2´´-*O*-rhamnoside isoprenyl, hexoside of methyl amentoflavone and dihydro-3',3'"-binaringen methyl ether) and compounds (protocatechuic acid hexoside, 1,3,5,6-tetrahydroxy-7-(3-methylbut-2-enyl) xanthone and 5,7,4´-tri-*O*-methyl quercetin) as potential antioxidant and antiarthritic candidates, respectively.

Conflicts of interest

The authors declare that they have no competing interests.

Acknowledgment

Authors would like to thank Dr. Sebaey Mahgoub for his contributions in UPLC-QTOF-MS/MS analysis of the methanolic extract of the plant leaves at The Proteomics and Metabolomics Research Program, Basic Research Department, the Children Cancer Hospital (CCHE-57357), Egypt.
(https://www.57357.org/en/department/proteomics-unit-dept/about-department/).

5. References

[1] Kingston DGI. Modern natural products drug discovery and its relevance to biodiversity conservation. J Nat Prod. 2010;74(3):496–511.
[2] Ritthiwigrom T, Laphookhieo S, Pyne SG. Chemical constituents and biological activities of *Garcinia cowa* Roxb. Maejo Int J Sci Technol. 2013;7:212–231.
[3] Mohamed AA, Habeeb HR, Azer SA. Survey, evaluation and documentation of the cultivated plants in Aswan Botanical Garden, Egypt. Bul Fac Agric Cairo. 2014;65:21–37.
[4] Nordstrom A, O'Maille G, Qin C, Siuzdak G. Nonlinear data alignment for UPLC-MS and HPLC-MS based metabolomics: quantitative analysis of endogenous and exogenous metabolites in human serum. Anal Chem. 2006;78:3289–3295.
[5] Konish Y, Kiyota T, Draghici C, Gao JM, Yeboah F, Acoca S, Jarussophon S, Purisima E. Molecular formula analysis by an MS/MS/MS method to expedite dereplication of natural products. Anal Chem. 2007;79:1187–1197.
[6] Zhou Y, Xu G, Choi FFK, Ding LS, Han QB, Song JZ, Qiao CF, Zhao QS, Xu HX. Qualitative and quantitative analysis of diterpenoids in *Salvia* species by liquid chromatography coupled with electrospray ionization quadrupole time-of-light tandem mass spectrometry. J Chromatogr., A 2009;1216(24):4847–4858.
[7] Mulholland DA, Mwangi EM, Dlova NC, Plant N, Crouch NR, Coombes PH. Non-toxic melanin production inhibitors from *Garcinia livingstonei* (Clusiaceae). J Ethnopharmacol. 2013;149(2):570–575.
[8] Kaikabo AA. Isolation and characterization of antibacterial compounds from a *Garcinia livingstonei* (Clusiaceae) leaf extract. A thesis submitted in fulfilment of the requirements for the degree of Magister Scientiae in Veterinary Science in the Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa. 2009
[9] Khumalo GP, Sadgrove NJ, Van Vuuren SF, Van Wyk BE. South Africa's best bark medicines prescribed at the Johannesburg Muthi markets for skin, gut, and lung infections: MIC's and brine shrimp lethality. Antibiotics (Basel) 2021;10(6):681.
[10] Al-Shagdari A, Alarcón AB, Cuesta-Rubio O, Piccinelli AL, Rastrelli L. Biflavonoids, Main Constituents from *Garcinia Bakeriana* Leaves. Nat Prod Commun. 2013;8(9):1237–40.
[11] Roux D, Hadi HA, Thoret DS, Guenard O, Thoison M, Pais T, Sevenet T. Structure activity relationship of polyisoprenyl benzophenones from *Garcinia pyrifera* on the tublin/microtubule system J Nat Prod. 2000;63:1070–1076.
[12] Yang NY, Han QB, Cao XW, Qiao CF, Song JZ, Chen SL, Yang DJ, Yiu H, Xu HX. Two new xanthones isolated from the stem bark of *Garcinia lancilimba*. Chem Pharm Bull. 2007;55(6):950–2.
[13] Jamila N, Khairuddean M, Khan SN, Khan N. Complete NMR assignments of bioactive rotameric (3–8) biflavonoids from the bark of *Garcinia hombroniana*. Magn Reson Chem. 2014;52(April):345–352.
[14] Muriithi E, Bojase-Moleta G, Majinda RRT. Benzophenone derivatives from *Garcinia livingstonei* and their antioxidant activities. Phytochem Lett. 2016;18:29–34.
[15] Priyadharisini J. Evaluation of *in vitro* antiarthritis activity of *Garcinia cambogia* fruit against the denaturation of albumin. J Med Pharm Allied Sci. 2019;8(I1):2059–2066.
[16] Evans WC. Trease and evans' pharmacognosy E-book. Elsevier Health Sciences. 2009
[17] Wojdyto A, Oszmianski J, Laskowski P. Polyphenolic compounds and antioxidant activity of new and old apple varieties. J Agric Food Chem. 2009;56:6520−6530.
[18] Mongkolsilp S, Pongbupakit I, Sae-Lee N, Sitthithaworn W. Radical scavenging activity and total phenolic content of medicinal plants used in primary health care. SWU J Pharm Sci. 2004;9(1):32–35.

[19] Fu M, Feng HJ, Chen Y, Wang DB, Yang, GZ. Antioxidant activity of *Garcinia xanthochymus* leaf, root and fruit extracts *in vitro*. Chin J Nat Med. 2012;10(2):129–134.
[20] Trisuwan K, Rukachaisirikul V, Phongpaichit S, Hutadilok-Towatana N. Tetraoxygenated xanthones and biflavanoids from the twigs of *Garcinia merquensis*. Phytochem Lett. 2013;6(4):511–513.
[21] Choudhury B, Kandimalla R, Elancheran R, Bharali R, Kotoky J. *Garcinia morella* fruit, a promising source of antioxidant and anti-inflammatory agents induces breast cancer cell death via triggering apoptotic pathway. Biomed Pharmacother. 2018;10:562–573.
[22] Acuna UM, Dastmalchi K, Basile MJ, Kennelly EJ. Quantitative high-performance liquid chromatography photo-diode array (HPLC-PDA) analysis of benzophenones and biflavonoids in eight *Garcinia* species. J Food Comp Anal. 2012;25:215–220.
[23] Tabit FT, Komolafe NT, Tshikalange TE, Nyila MA. Phytochemical constituents and antioxidant and antimicrobial activity of selected plants used traditionally as a source of food. J. Med. Food 2016;19(3):324–329.
[24] Joseph KS, Bolla S, Joshi K, Bhat M, Naik K, Patil S, Bendre S, Gangappa B, Haibatti V, Payamalle S, Shinde S, Dewir YH, Murthy HN. Determination of chemical composition and nutritive value with fatty acid compositions of African mangosteen (*Garcinia Livingstonei*). Erwerbs-Obstbau. 2017;59:195–202.
[25] O'Dell JR. Therapeutic strategies for rheumatoid arthritis. N Engl J Med. 2004;350:2591–602.
[26] Tripathi KD. Essentials of medical pharmacology. 6th edition Jaypee Brothers medical Publishers (P) LTD. 2006;185.
[27] Sheelarani T, Gopal V, Seethalakshmi S, Chitra K. *In vitro* anti inflammatory and anti arthritic activity of selected medicinal plant. Int J Pharm Sci Rev Res. 2014;28(2):162–163.
[28] Elmaaty AA, Darwish KM, Chrouda A, Boseila AA, Tantawy MA, Elhady SS, Shaik AB, Mustafa M, Al-Karmalawy AA. In Silico and *in vitro* studies for benzimidazole anthelmintics repurposing as VEGFR-2 Antagonists: Novel mebendazole-loaded mixed micelles with enhanced dissolution and anticancer activity. ACS Omega 2022;7(1):875–899.
[29] El-Masry RM, Al-Karmalawy AA, Radwan A, Mahmoud SH, Mostafa A, Kadry HH, Abou-Seri SM, Taher AT. Newly synthesized series of oxoindole–oxadiazole conjugates as potential anti-SARS-CoV-2 agents: *in silico* and *in vitro* studies. New J Chem. 2022;46(11):5078–5090.
[30] Kumazawa S, Taniguchi M, Suzuki Y, Shimura M, Kwon MS, Nakayama T. Antioxidant activity of polyphenols in carob pods. J Agric Food Chem. 2002;50:373–377.
[31] Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in Propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10:178–182.
[32] Boly R, Lamkami T, Lompo M, Dubois J, Guissou IP. DPPH free radical scavenging activity of two extracts from *Agelanthus dodoneifolius* (*Loranthaceae*) leaves. Int J Toxicol Pharmacol Res. 2016;8:29–34.
[33] Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal Biochem. 1996;239:70-76.
[34] Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution of total antioxidant activity. Food chem. 2001;73:239–244.
[35] Chen Z, Bertin R, Froldi G. EC_{50} estimation of antioxidant activity in DPPH_ assay using several statistical programs. Food Chem. 2013;138:414–420.
[36] Singh S, Sharma N. Evaluation of *in vitro* anti arthritic activity of *acacia auriculiformis* A. Cunn. Ex. Benth. Stem bark. World J Pharm Pharm Sci. 2016;5(2):1659–1664.
[37] Shinde U, Phadke A, Nari AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Membrane stabilization activity a possible mechanism of action for the anti-inflammatory activity of Cedrusde odara wood oil. Fitoterapia. 1999;70(3):251–257.
[38] Kong LD, Abliz Z, Zhou CX, Li LJ, Cheng CH, Tan RX. Glycosides and xanthine oxidase inhibitors from *Conyza bonariensis*. Phytochem. 2001;58(4):645–51.
[39] Mohammad MK, Almasri IM, Tawaha K, Isaa A, Al-Nadaf A, Hudaib M, Al-Khatib HS, Abu-Gharbieh E, Bustanji Y. Antioxidant, antihyperuricemic and xanthine oxidase inhibitory activities of *Hyoscyamus reticulatus*. Pharm Biol. 2010;48:1376–83.
[40] Huey R., Morris GM, Forli S. Using AutoDock 4 and AutoDock vina with AutoDockTools: A tutorial. The Scripps Research Institute Molecular Graphics Laboratory 2012;10550(92037):p. 1000.
[41] Yuan S, Chan HS, Hu Z. Using PyMOL as a platform for computational drug design. Wiley Interdisciplinary Reviews: Comput Mol Sci. 2017;7(2):e1298.
[42] Taher RF, Al-Karmalawy AA, Abd-El Maksoud AI, Khalil H, Hassan A, El-Khrisy EA, El-Kashak W. Two new flavonoids and anticancer activity of Hymenosporum flavum:in vitro and molecular docking studies. J Herbmed Pharmacol. 2021;10(4):443–458.
[43] Mahmoud D.B, Ismail WM, Moatasim Y, Kutkat O, El-Meshad AN, Ezzat SM, El-Deeb KS, El-Fishawy AM, Gomaa MR, Kandeil A, Al-Karmalawy AA, Ali MA, Mostafa A. Delineating a potent antiviral activity of Cuphea ignea extract loaded nano-formulation against SARS-CoV-2:In silico and in vitro studies. J Drug Deliv Sci Technol. 2021;66:102845.
[44] Fernández-Fernández R, López-Martínez JC, Romero-González R, Martínez-Vidal JL, Alarcón Flores MI, Garrido Frenich A. Simple LC–MS determination of citric and malic acids in fruits and vegetables. Chroma. 2010;72(1-2):55–62.
[45] Carrillo-Hormaza L, Ramírez AM, Quintero-Ortiz C, Cossio M, Medina S, Ferreres F, Gil-Izquierdo A, Osorio E. Comprehensive characterization and antioxidant activities of the main biflavonoids *of Garcinia madruno*: A novel tropical species for developing functional products. J Funct Foods. 2016;27:503–516.

[46] Peng H, Deng Z, Chen X, Sun Y, Zhang B, Li H. Major chemical constituents and antioxidant activities of different extracts from the peduncles of *Hovenia acerba* Lindl. Int J Food Prop. 2018;21(1):2135–2155.

[47] Su H, Li X, Li Y, Kong Y, Lan J, Huang Y, Liu Y. Chemical profiling and rapid discrimination of *Blumea riparia* and *Blumea megacephala* by UPLC-Q-Exactive-MS/MS and HPLC. Chinese Herb Med. 2022;15(2):317–328.
[48] Jena BS, Jayaprakasha GK, Sakariah KK. Organic acids from leaves, fruits and rinds of *Garcinia cowa.* J Agric Food Chem. 2002;50(12):3431–3434.
[49] Parthasarathy U, Nandakishore OP, Kumar RS, Babu KN, Zachariah TJ, Parthasarathy VA. Chromatographic fingerprinting and estimation of organic acids in selected *Garcinia* species. Int J Innov Hortic. 2012;1(1):68–73.
[50] Rahman MM, Kim MJ, Kim JH, Kim SH, Go HK, Kweon MH, Kim DH. Desalted *Salicornia europaea* powder and its active constituent, trans-ferulic acid, exert anti-obesity effects by suppressing adipogenic-related factors. Pharm Biol. 2018; 56(1):183–191.
[51] Chkhikvishvili I, Sanikidze T, Gogia N, Mchedlishvili T, Enukidze M, Machavariani M, Vinokur Y, Rodov V. Rosmarinic acid-rich extracts of summer savory (*Satureja hortensis* L.) protect jurkat t cells against oxidative stress. Oxid Med Cell Longev. 2013;2013:1–9.
[52] Gaya M, Repetto V, Toneatto J, Anesini C, Piwien-Pilipuk, G Moreno S. Antiadipogenic effect of carnosic acid, a natural compound present in *Rosmarinus officinalis*, is exerted through the C/EBPs and PPARγ pathways at the onset of the differentiation program. Biochim Biophys Acta. 2013;1830:3796–3806.
[53] Zhao Y, Li X, Zeng X, Huanga S, Houa S, Lai X. Characterization of phenolic constituents in *Lithocarpus polystachyusI.* J Anal Methods**.** 2014; 6**:**1359–1363.
[54] Benchikh Y, Paris C, Louaileche H**,** Charbonnel C, Ghou M, Chebi L. Comparative characterization of green and ripe carob (*Ceratonia siliqua* L.): physicochemical attributes and phenolic profile. SDRP J Food Sci Technol. 2016;1(3):1–7.
[55] Bouhafsoun A, Yilmaz MA, Boukeloua A, Temel H, Harche MK. Simultaneous quantification of phenolic acids and flavonoids in *Chamaerops humilis* L. using LC–ESI-MS/MS. Food Sci Technol. 2018;38:242–247.
[56] Zadernowski R, Czaplicki S, Naczk M. Phenolic acid profiles of mangosteen fruits (*Garcinia mangostana*). Food Chem. 2009;112(3):685–689.
[57] Singh P, Roy TK, Kanupriya C, Tripathi PC Kumar P, Shivashankara KS. Evaluation of bioactive constituents of *Garcinia indica* (kokum) as a potential source of hydroxycitric acid, anthocyanin, and phenolic compounds. LWT 2022;156:112999.
[58] Manuja R, Sachdeva S, Jain A, Chaudhary J. A comprehensive review on biological activities of p-hydroxy benzoic acid and its derivatives. Int J Pharm Sci Rev Res. 2013:22:109–115.
[59] Masella R, Santangelo C, D'Archivio M, Li Volti G, Giovannini G, Galvano F. Protocatechuic acid and human disease prevention:biological activities and molecular mechanisms. *Curr Med Chem.* 2012;19(18):2901–2917.
[60] Borges A, Ferreira C, Saavedra MJ, Simões M. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microb Drug Resist.* 2013;19(4):256–265.
[61] Asnaashari M, Farhoosh R, Sharif A. Antioxidant activity of gallic acid and methyl gallate in tri-acylglycerols of Kilka fish oil and its oil-in-water emulsion. Food Chem. 2014;15(159):439–444.
[62] Du XG, Wang W, Zhang QZ, Cheng J, Avula B, Khan IA, Guo DA. Identification of xanthones from *Swertia punicea* using high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom. 2012;26:2913–2923.
[63] Hay AE, He´lesbeux JJ, Duval O, Labaïed M, Philippe Grellier P, Richomme P. Antimalarial xanthones from *Calophyllum caledonicum* and *Garcinia vieillardii*. Life Sci. 2004;75:3077– 3085.
[64] Urbain A, Marston A, Queiroz EF, Ndjoko K, Hostettmann K. Xanthones from *Gentiana campestrisas* new acetylcholinesterase inhibitors. Planta Med. 2004;70(10):1011–1014.
[65] Duangsrisai S, Choowongkomon K, Bessa L, Costa P, Amat N, Kijjoa A. Antibacterial and EGFR-tyrosine kinase inhibitory activities of polyhydroxylated xanthones from *Garcinia succifolia*. Molecules. 2014;19(12):19923–19934.
[66] Li H, Meng X, Zhang L, Zhang B, Liu X, Fu W, Tan H, Lao Y, Xu H. Oblongifolin C and guttiferone K extracted from *Garcinia yunnanensis* fruit synergistically induce apoptosis in human colorectal cancer cells *in vitro.* Acta Pharmacol Sin. 2017;38(2):252–263.
[67] Aberham A, Schwaiger S, Stuppner H, Ganzera M. Quantitative analysis of iridoids, secoiridoids, xanthones and xanthone glycosides in *Gentiana lutea* L. roots by RP-HPLC and LC–MS. J Pharm Biomed Anal. 2007:45(3): 437–442.
[68] Yang H, Figueroa M, To S, Baggett S, Jiang B, Basile MJ, Weinstein IB, Kennelly EJ. Benzophenones and biflavonoids from *Garcinia livingstonei* fruits. J Agric Food Chem. 2010;58(8):4749–4755.
[69] Zhou Y, Han QB, Song JZ, Qiao CF, Xu HX. Characterization of polyprenylated xanthones in *Garcinia xipshuanbannaensis* using liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. J Chromatogr. A 2008;1206(2):131–139.
[70] Tian Z, Shen J, Moseman AP, Yang Q, Yang J, Xiao P, Wu E, Kohane IS. Dulxanthone A induces cell cycle arrest and apoptosis via up-regulation of p53 through mitochondrial pathway in HepG2 cells. Int J Cancer. 2008:122(1):31–8.
[71] Ha NTT, Van Cuong P, Anh LTT, Tra NT, Cham BT, Son N. T. Antimicrobacterial xanthones from *Garcinia mackeaniana* leaves. Vietnam J Chem. 2020;58(3):343–348.
[72] Castañeta G, Cifuentes N, Sepulveda B, Bárcenas-Pérez D, Cheel J, Areche C. Untargeted metabolomics by using UHPLC–ESI–MS/MS of an extract obtained with ethyl lactate green solvent from *Salvia rosmarinus.* Separations. 2022;9:327–339.
[73] Zhang Z, Jia P, Zhang X, Zhang Q, Yang H, Shi H, Zhang L. LC–MS/MS determination and pharmacokinetic study of seven flavonoids in rat plasma after oral administration of *Cirsium japonicum* DC. Extract. J Ethnopharmacol. 2014;158 Pt A:66–75.

[74] da Silva Mathias M, Rodrigues de Oliveira R. Differentiation of the phenolic chemical profiles of *Cecropia pachystachya* and *Cecropia hololeuca*. Phytochem Anal. 2019;30(1):73–82.
[75] Qiu XL, Zhang QF. Chemical profile and pancreatic lipase inhibitory activity of *Sinobambusa tootsik* (Sieb.) Makino leaves. Peer J. 2019;23(7):e7765.
[76] Pereira CAM, Yariwake JH and Mc Cullagh M. Distinction of the C-glycosylflavone isomer pairs orientin/isoorientin and vitexin/isovitexin using HPLC-MS exact mass measurement and in-source CID. Phytochem Anal. 2005;16(5):295–301.
[77] Küpeli E, Aslan M, Gürbüz I, Yesilada E. Evaluation of *in vivo* biological activity profile of isoorientin. Z Naturforsch C J Biosci. 2004;59(11-12):787–790.
[78] Rukachaisirikul V, Saelim S, Karnsomchoke P, Phongpaichit S. Friedolanostanes and lanostanes from the leaves of *Garcinia hombroniana*. J Nat Prod. 2005;68:1222-1225.
[79] Peng X, Zheng Z, Cheng KW, Shan F, Ren GX, Chen F, Wang M. Inhibitory effect of mung bean extract and its constituents vitexin and isovitexin on the formation of advanced glycation endproducts. Food Chem. 2008;106(2):475–481.
[80] Pattamadilok D, Niumsakul S, Limpeanchob N, Ingkaninan K, Wongsinkongman P. Chemical constituents and anti-hyperlipidemic activity of *Garcinia cowa* leaves. J Thai Trad Alt Med. 2010;8(2/3):152–160.
[81] Lam KY, Ling APK, Koh RY, Wong YP and Say YH. A review on medicinal properties of orientin. Adv Pharmacol Sci. 2016;1–9.
[82] Wang Y, Ni W, Jin X, Li J, Yu Y. Vitexin-2-O-rhamnoside improves immunosuppression, oxidative stress, and phosphorylation of PI3K/Akt signal pathway in cyclophosphamide treated mice. Eur J Pharmacol. 2022;925:174999.
[83] Tsimogiannis D, Samiotaki M, Panayotou G and Oreopoulou V. Characterization of flavonoid subgroups and hydroxy substitution by HPLC-MS/MS. Molecules. 2007;12:593–606.
[84] Pinheiro PF, Justino GC. Structural analysis of flavonoids and related compounds - A review of spectroscopic applications. In Phytochemicals, A Global Perspective of Their Role in Nutrition and Health 2012;33–56.
[85] Ads EN, Hassan SI, Rajendrasozhan S, Hetta MH, Aly SH, Ali MA. Isolation, structure elucidation and antimicrobial evaluation of natural pentacyclic triterpenoids and phytochemical investigation of different fractions of *Ziziphus spina-christi* (L.) stem bark using LCHRMS analysis. *Molecules.* 2022:27(6):1805.
[86] Patel K, Patel DK. Medicinal importance, pharmacological activities, and analytical aspects of hispidulin:A concise report. J Tradit Complement Med. 2016;7(3):360–366.
[87] Li H, Wei YY, Li XH, Zhang SS, Zhang RT, Li JH, Ma BW, Shao SB, Lv ZW, Ruan H, Zhou HG, Yang C. Diosmetin has therapeutic efficacy in colitis regulating gut microbiota, inflammation, and oxidative stress via the circ-Sirt1/Sirt1 axis. Acta Pharmacol Sin. 2022;43(4):919–932.
[88] Li P, AnandhiSenthilkumar H, Wu S, Liu B, Guo Z, Fata JE, Kennelly EJ, Long C. Comparative UPLC-QTOF-MS-based metabolomics and bioactivities analyses of *Garcinia oblongifolia*. J Chromatogr. B 2016;1011:179–195.
[89] Aravind APA, Pandey R, Kumar B, Asha KRT, Rameshkumar KB. Phytochemical screening of *Garcinia travancorica* by HPLC-ESI-QTOF mass spectrometry and cytotoxicity studies of the major biflavonoid fukugiside. Nat Prod Commun. 2016;11(12):1839–1842.
[90] Islam A, Islam MS, Rahman MK, Uddin MN, Akanda MR. The pharmacological and biological roles of eriodictyol. Arch Pharm Res. 2020;43(6):582–592.
[91] Chiang YM, Kuo YH, Oota S, Fukuyama Y. Xanthones and benzophenones from the stems of *Garcinia multiflora*. J Nat Prod. 2003;66(8):1070–1073.
[92] Bodet C, La VD, Epifano F, Grenier D. Naringenin has anti-inflammatory properties in macrophage and ex vivo human whole-blood models. J Periodontal Res. 2008;43:400–407.
[93] Materska M. Quercetin and its derivatives: Chemical structure and bioactivity – A review. *Polish J Food* Nutr Sci. 2008;58(4):407–413.
[94] Cavia-Saiz M, Busto MD, Pilar-Izquierdo MC, Ortega N, Perez-Mateos M, Muniz P. Antioxidant properties, radical scavenging activity and biomolecule protection capacity of flavonoid naringenin and its glycoside naringin:A comparative study. J Sci Food Agric. 2010;90(7):1238–44.
[95] Buba CI, Okhale SE, Muazzam I. *Garcinia kola*: The phytochemistry, pharmacology and therapeutic applications. Inter J Pharmacogn. 2016;3(2):67-81.
[96] Eid HM and Haddad PS. The antidiabetic potential of quercetin: underlying mechanisms. Curr Med Chem. 2017;24(4):355–364.
[97] Lei X, Guo J, Wang Y, Cui J, Feng B, Su Y. Inhibition of endometrial carcinoma by kaempferol is interceded through apoptosis induction, G2/M phase cell cycle arrest, suppression of cell invasion and up regulation of m-TOR/PI3K signalling pathway. J BUON. 2019;24:1555–1561.
[98] Ali A, Cottrell JJ, Dunshea FR. LC-MS/MS characterization of phenolic metabolites and their antioxidant activities from Australian native plants. Metabolites 2022;12:1016.
[99] Baranwal A, Aggarwal P, Rai A, Kumar N. Pharmacological actions and underlying mechanisms of catechin: A Review. Mini Rev Med Chem. 2022;22(5):821–833.
[100] Suzart LR, Daniel JFS, Carvalho MG, Coelho MA. Biodiversidade flavonoídica e aspectos farmacológicos em espécies dos gêneros Ouratea e Luxemburgia (Ochnaceae). Quimica Nova. 2007;30(4):984–987.
[101] Yao H, Chen B, Zhang Y, Ou H, Li Y, Li S, Shi P, Lin X. Analysis of the total biflavonoids extract from *Selaginella doederleinii* by HPLC-QTOF-MS and its *in vitro* and *in vivo* anticancer effects. Molecules. 2017;22:325–342.
[102] Pandey R, Chandra P, Kumar B, Srivastva M, Aravind AP, Shameer PS, Rameshkumar KB. Simultaneous determination of multi-class bioactive constituents for quality assessment of *Garcinia* species using UHPLC–QqQ LIT –MS/MS. Ind Crops Prod. 2015;77:861–872.

[103] On S, Aminudin N, Ahmad F, Sirat H M, Taher M. Chemical constituents from stem bark of *Garcinia prainiana* and their bioactivities. Int J Pharmacogn Phytochem Res. 2016;8(5):756–760.
[104] Castardo JC, Prudente AS, Ferreira J, Guimarães CL, Monache FD, Filho VC, Otuki MS, Cabrini DA. Anti-inflammatory effects of hydroalcoholic extract and two biflavonoids from *Garcinia gardneriana* leaves in mouse paw oedema. J Ethnopharmacol. 2008;118(3):405–411.
[105] Osorio E, Londoño J, Bastida J. Low-density lipoprotein (LDL)-antioxidant biflavonoids from *Garcinia madruno*. Molecules. 2013;18(5):6092–6100.
[106] Naves VML, dos Santos MH, Ribeiro IS, da Silva CA, Silva NC, da Silva MA, da Silva GA, Dias ALT, Ionta M, Dias DF. Antimicrobial and antioxidant activity of *Garcinia brasiliensis* extracts. S Afr J Bot. 2019;124:244–250.
[107] Konziase B. Protective activity of biflavanones from *Garcinia kola* against *Plasmodium* infection. J Ethnopharmacol. 2015;22(172):214–218.
[108] Tshibangu PT, Kapepula PM, Kapinga MJK, Lupona HK, Ngombe NK, Kalenda DT, Jansen O, Jansen JN, Tits M, Angenot L, Rozet E, Hubert P, Marini RD, Frédérich M. Fingerprinting and validation of a LC-DAD method for the analysis of biflavanones in *Garcinia kola*-based antimalarial improved traditional medicines. J Pharm Biomed Anal. 2016;128:382–390.
[109] Recalde-Gil MA, Klein-Júnior LC, Passos CDS, Salton J, Bordignon SAL, Monace FD, Cechinel V, Henriquesa AT. Monoamine oxidase inhibitory activity of biflavonoids from branches of *Garcinia gardneriana* (Clusiaceae). Nat Prod Commun. 2017;12(4):505–508.
[110] Abdullah I, Phongpaichit S, Voravuthikunchai SP, Mahabusarakam W. Prenylated biflavonoids from the green branches of *Garcinia dulcis*. Phytochem Lett. 2018;23:176–179.
[111] Zhang YX, Li QY, Yan LL, Shi Y. Structural characterisation and identification of biflavones in *Selaginella tamariscina* by liquid chromatography-diode-array detection/electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom. 2011;25:2173–2186.
[112] Ma SC, But PPH, Ooi VEC, He YH, Lee SHS, Lee SF, Lin RC. Antiviral amentoflavone from *Selaginella sinensis*. Bio Pharm Bull. 2001;*24:*311–312.
[113] Shan L, Wu Y, Yuan L, Zhang Y, Xu Y, Li Y. Rapid screening of chemical constituents in *Rhizoma anemarrhenae* by UPLC-Q-TOF/MS combined with data post processing techniques. Evid. Based Complement. Alternat Med. 2017;2017:1–14.
[114] Kumar V, Sood H, Chauhan RS. Detection of intermediates through high-resolution mass spectrometry for constructing biosynthetic pathways for major chemical constituents in a medicinally important herb, *Swertia chirayita.* Nat Prod Res. 2015;29(15):1449–1455.
[115] Jantan I, Saputri FC. Benzophenones and xanthones from *Garcinia cantleyana var. cantleyana* and their inhibitory activities on human low-density lipoprotein oxidation and platelet aggregation. Phytochem. 2012;80:58–63.
[116] Kuete V, Tchakam PD, Wiench B, Ngameni B, Wabo HK, Tala MF, Moungang ML, Ngadjui BT, Murayama T, Efferth T. Cytotoxicity and modes of action of four naturally occuring benzophenones:2,2′,5,6′-Tetrahydroxybenzophenone, guttiferone E, isogarcinol and isoxanthochymol. Phytomed. 2013;20(6):528–536.
[117] Lee YJ, Lee SY. Maclurin exerts anti-cancer effects in human osteosarcoma cells via prooxidative activity and modulations of PARP, *p38, and ERK signaling.* IUBMB Life. 2021;73(8):1060–1072.
[118] Zhou Y, Lee S, Choi FFK, Xu G, Liu X, Song JZ, Li SL, Qiao CF, Xu HX. Qualitative and quantitative analysis of polycyclic polyprenylated acylphloroglucinols from Garcinia species using ultra performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. Anal Chim Acta. 2010;678: 96–107.
[119] Kaur R, Chattopadhyay SK, Tandon S, Sharma S. Large scale extraction of the fruits of *Garcinia indica* for the isolation of new and known polyisoprenylated benzophenone derivatives. Ind Crops Prod. 2012;37(1):420–426.
[120] Fu W, Wu M, Zhu L, Lao Y, Wang L, Tan H, Yuan Q, Xu H. Prenylated benzoylphloroglucinols and biphenyl derivatives from the leaves of *Garcinia multiflora* Champ. RSC Adv. 2015;5(95):78259–78267.
[121] Piccinelli AL, Campone L, Piaz FD, Cuesta-Rubio O, Rastrelli L. Fragmentation pathways of polycyclicpolyisoprenylated benzophenones and degradation profile of nemorosone by multiple-stage tandem mass spectrometry. J Am Soc Mass Spectrom. 2009;20(9):1688 –1698.
[122] Aboobaker VS, Balgi AD, Bhattacharya RK. *In vivo* effect of dietary factors on the molecular action of aflatoxin B1: role of non-nutrient phenolic compounds on the catalytic activity of liver fractions. In vivo 1994;8(6):1095–8.
[123] Weng JR, Lin CN, Tsao LT, Wang JP. Terpenoids with a new skeleton and novel triterpenoids with anti-inflammatory effects from *Garcinia subelliptica*. Chem Eur J. 2003;9(22):5520–5527.
[124] Rahman MM, Islam MB, Biswas M, Khurshid AH. *In vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. BMC Res. Notes 2015;8(1):1–9.
[125] Kusmardiyani S, Novita G, Fidrianny I. Antioxidant activities from various extracts of different parts of kelakai (*Stenochlaena palustris*) grown in Central Kalimantan – Indonesia. Asian J Pharm Clin Res. 2016;9(2):215–219.
[126] Shah P, Modi HA. Comparative study of DPPH, ABTS and FRAP assays for determination of antioxidant activity. Int J Res App Sci Eng Tech. (IJRASET) 2015;3(VI):636–641.
[127] Ramallo IA, Zacchino SA, Furlan RL. A rapid TLC autographic method for the detection of xanthine oxidase inhibitor and superoxide scavengers. Phytochem Anal. 2006;17(1):15–9.
[128] Virsaladze DK, Tetradze LO, Dzhavashvili LV, Esaliia NG, Tananashvili DE. Levels of uric acid in serum in patients with metabolic syndrome. Georgian. Med News. 2007;(146):35–7.
[129] Azeem AK, Dilip C, Prasanth SS, Junise HSV, Kumar S, Naseera C. Anti-inflammatory activity of the glandular extracts of *Thunnus alalunga*. Asian Pac J Trop Med. 2010; 3(10):412–420.
[130] Kishore G, Siva G, Sindhu ES. *In Vitro* Anti-inflammatory and anti-arthritic activity of leaves of *Physalis angulata* L. Int J Pharm Ind Res. 2011;1:211–213

[131] da Fonseca LJS, Nunes-Souza V, Goulart MOF, Rabelo LA. Oxidative stress in rheumatoid arthritis: what the future might hold regarding novel biomarkers and add-on therapies. Oxid Med Cell Longev. 2019:1–16.
[132] Cimen MYB, Cimen O¨B, Kacmaz M, Ozturk HSO, Yorgancioglu R, Durak I. Oxidant/antioxidant status of the erythrocytes from patients with rheumatoid arthritis. Clin Rheumatol. 2000;19:275–277
[133] Warriar P, Barve K, Prabhakar B Anti-arthritic effect of garcinol enriched fraction against adjuvant induced arthritis. Recent Pat. Inflamm. Allergy Drug Discov. 2019;13(1):49–56.