



OPEN *Eucalyptus torquata* L. flowers: a comprehensive study reporting their metabolites profiling and anti-gouty arthritis potential

Rehab M. S. Ashour^{1,5}, Riham A. El-Shiekh^{1,5}, Mansour Sobeh², Mohamed A. O. Abdelfattah³, Marwa M. Abdel-Aziz⁴ & Mona M. Okba^{1✉}

Gouty arthritis is one of the most common metabolic disorders affecting people. Plant based drugs can lower the risk of this health disorder. The anti-gouty potential of *Eucalyptus torquata* flowers methanol extract (ETME) was evaluated in vitro via measuring the inhibitory effects of five pro-inflammatory enzymes; xanthine oxidase (XO), hyaluronidase, lipoxygenase (5-LOX), cyclooxygenases COX-1, and COX-2, in addition to evaluating the inhibition of histamine release, albumin denaturation, membrane stabilization, tyrosinase, and protease inhibitory activities. Also, its antioxidant potential was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assays and ferric reducing power assay (FRAP). HPLC-PDA-MS/MS was used to identify the metabolites in the tested extract. The latter exhibited substantial anti-arthritic properties in all assays with comparable potential to the corresponding reference drugs. HPLC-MS/MS analysis of this bioactive extract tentatively annotated 46 metabolites including phloroglucinols, gallic and ellagic acids derivatives, terpenes, flavonoids, fatty acids, and miscellaneous metabolites. Our study highlights the medicinal importance of *E. torquata* as an anti-gouty candidate and opens new avenues of gouty management.

Gout, also known as gouty arthritis, is a common disease characterized by hyperuricemia, abnormally high level of blood uric acid that leads to the accumulation of monosodium urate crystals in joints causing inflammatory arthritis^{1,2}. Gouty arthritis has been associated with the development of other several disorders including cancer, obesity, hyperlipidemia, hypertension, and diabetes³. Non-steroidal anti-inflammatory drugs and colchicine are typically utilized in gout treatment, however they come with several adverse effects that impose high demand for safer alternatives⁴.

Several medicinal plants and phytochemicals play a major role in the treatment of a wide array of diseases and pathological conditions, among them oxidative stress related disorders^{5–11}. High levels of oxygen reactive species and free radicals end up in a state of cellular oxidative stress, which mediate acute inflammation through cytokines stimulation and pro-inflammatory enzymes activation, such as lipoxygenase, cyclooxygenase, hyaluronidase, tyrosinase and xanthine oxidase^{12,13}. Also, denaturation of proteins, membrane stabilization and protease inhibitory activity are well documented causes for inflammation and rheumatoid arthritis. In addition, histamine is well reported as a potent inflammatory mediator¹⁴. Therefore, the discovery of natural secondary metabolites with pro-inflammatory enzymes inhibiting activity along with potent antioxidant potential would be highly beneficial in controlling and combating gouty arthritis.

Eucalyptus (family Myrtaceae) is a well-known genus that is traditionally used to treat a wide array of disorders¹⁵. *Eucalyptus* plants accumulate various classes of secondary metabolites including phloroglucinols, flavonoids, essential oils, tannins, triterpenes, and oleuropeic acid derivatives^{9,16} with a broad spectrum of pharmacological activities, such as antibacterial, antioxidant, anticancer, antiseptic, and anti-inflammatory potentials^{15,17}. *E. torquata* Luehm, known as coral gum, is an endemic tree of Western Australia. Its leaves, stems, and flowers furnished promising antibacterial, antifungal, and anticancer activities¹⁸.

¹Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt. ²Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany. ³College of Engineering and Technology, American University of the Middle East, Egaila 54200, Kuwait. ⁴Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo 11651, Egypt. ⁵These authors contributed equally: Rehab M. S. Ashour and Riham A. El-Shiekh. ✉email: mona.morad@pharma.cu.edu.eg

The aim of this study is to evaluate the potential of *E. torquata* flowers methanol extract in the management of gouty arthritis, by testing its inhibitory activity against several key enzymes related to gout and inflammation. These include xanthine oxidase, hyaluronidase, 5-LOX, COX-1, COX-2, tyrosinase, and protease enzymes. In addition, the potential of *E. torquata* to affect histamine release, albumin denaturation, and membrane stabilization will be explored. Moreover, HPLC–MS/MS will be used to tentatively annotate the secondary metabolites composition of ETME.

Material and methods

Plant material and extraction

Eucalyptus torquata Luehm. flowers were collected in May 2019 from El-Kobba palace, Cairo, Egypt. Mrs. Therese Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture, Cairo, Egypt authenticated the plant. A voucher specimen (No. 05.06.19.II) was deposited in the Pharmacognosy Department, Faculty of Pharmacy, Cairo University Museum. The air-dried flowers were ground, extracted by maceration in methanol. The combined methanol extract was concentrated under reduced pressure at temperature not exceeding 50 °C till dryness. Collection of the plant material, complied with the national, institutional, and international legislations and guidelines.

Chemicals

Methanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), ascorbic acid, hyaluronic acid (human umbilical cord), sodium hydroxide, hyaluronidase (bovine testes), calcium chloride, *p*-dimethylaminobenzaldehyde (PDMAB), and sodium borate were purchased from Sigma-Aldrich (USA). COX (ovine) and LOX inhibitor screening assay kits (Cayman Chemical Company, MI, USA), 96-well cell culture plate (Corning Life Sciences, Lowell, MA, USA), PMA (Sigma-Aldrich, St. Luis, MO, USA), and histamine release assay kit (SPI-Bio, France) were used.

Biological study

In vitro antioxidant activity

DPPH radical scavenging, ABTS radical scavenging and FRAP assays were done according to Dudonne et al.¹⁹ method minor modifications. The details are mentioned in the supplementary file.

In vitro anti-inflammatory and anti-arthritic activities

COX-1, COX-2, and LOX inhibitory activity

The COX inhibitory activity was assayed using Cayman colorimetric COX (ovine) inhibitor screening assay kit, according to the manufacturer's instructions. The selectivity index (S.I.) was then calculated as $IC_{50} (COX-1) / IC_{50} (COX-2)$. *In vitro* 5-lipoxygenase (5-LOX) inhibitory assay was carried out using lipoxygenase inhibitor screening assay kit, according to the manufacturer's instructions. Diclofenac sodium was used as a reference drug.

Albumin denaturation, membrane stabilization and protease inhibitory activities

Albumin denaturation inhibition was evaluated using the previously described method²⁰. Membrane stabilization and proteinase inhibitory potentials were evaluated using the previously reported method²¹. Diclofenac was used as the reference drug. All the experiments were performed in triplicate.

Xanthine oxidase (XO) inhibitory activity

The XO inhibitory activity was assayed following the protocol reported in²². Detailed procedures are described in the supplementary file.

Tyrosinase inhibitory activity

Tyrosinase inhibition assay was performed with L-DOPA as substrate adopting the previously described method²³. Detailed procedures are described in the supplementary file.

Hyaluronidase inhibitory activity

Hyaluronidase inhibitory activity of ETME was evaluated spectrophotometrically as reported by Perera et al.²⁴ with minor modifications. Detailed procedures are described in the supplementary file.

Histamine release inhibitory activity

U937 human monocytes (ATCC, Manassas, VA, USA) were used to study the effect of the extract on histamine release. U937 cells (50,000 cells) were plated in a 96-well plate and treated with various concentration (1000–7.81 µg/mL) of the extract, in presence or absence of 20 nM phorbol myristate acetate (PMA) for 1 h. The cell culture supernatants collected from either untreated control or treated cultures were centrifuged at 10,000 g for 5 min at 4 °C and assessed for the released histamine by the commercially available ELISA kit. Diclofenac was used as the positive control.

Phytochemical analysis

HPLC–PDA-MS was used to identify the phytoconstituents of ETME using ThermoFinnigan LCQ–Duo ion trap mass spectrometer (ThermoElectron Corporation, Waltham, MA, USA) with an ESI source (ThermoQuest Corporation, Austin, TX, USA)^{25,26}. Details of the HPLC system are described in the supplementary file.

Virtual screening and drug likeness analysis

Molecular modeling was done via docking the phytoconstituents of ETME to cyclooxygenase 1 (COX-1, PDB code: 5WBE), cyclooxygenase 2 (COX-2, PDB code: 5IKR), 5-lipoxygenase (5-LOX, PDB code: 3V99), and xanthine oxidase (XO, PDB code: 3NVY) using MOE software (MOE2022.v11.18.1) as previously described by Sobeh et al.²⁷ to virtually screen the binding mode of the extract's components in the aforementioned enzymes active site. Moreover, drug likeness analysis was performed by calculating the five molecular descriptors associated with drug likeness and oral bioavailability using the QSAR tool of MOE software.

Results

Gouty arthritis is one of the most common metabolic complaints affecting patients worldwide. Our study traced the potential use of *E. torquata* flowers for the management of hyperuricemia and gout, in correlation with its phytochemical profile. Scheme for extraction, biological evaluation, and chemical analysis of ETME is presented in Fig. 1.

Biological study

In vitro antioxidant activity

ETME exhibited potent antioxidant activity in comparison to ascorbic acid, as revealed by three different assays: DPPH, FRAP, and ABTS, Table 1.

In vitro anti-inflammatory and anti-arthritis activities of *E. torquata* flower methanol extract (ETME)

ETME inhibited COX-1, COX-2 and 5-LOX with comparable potential to the reference drugs. It also showed excellent selectivity towards COX-2 enzyme, Table 1. Anti-gouty arthritic activity of ETME was tested *in vitro* via evaluating its potential towards albumin denaturation, membrane stabilization, histamine release, and its inhibitory potential against protease, xanthine oxidase, tyrosinase, and hyaluronidase enzymes. ETME showed very promising activity in all tested assays, Table 1.

Phytochemical analysis

The HPLC–MS analysis of ETME allowed the tentative identification of 46 plant metabolites belonging to several phytochemical classes. These include phloroglucinols, gallic and ellagic acid derivatives, terpenes, flavonoid glycoside, fatty acids, and miscellaneous metabolites. Each metabolite observed molecular weight, fragment ions, and its identity is recorded in Table 2. Identification of the peaks were based on comparing their spectral masses with the data reported in the previous literature on Genus *Eucalyptus*^{6,9,16}.

Results revealed the high abundance of **phloroglucinols** in ETME; 34 phloroglucinols. Eighteen phloroglucinol-sesquiterpene adducts were tentatively identified. Out of which, five signals with fragmentation pattern; m/z 471, m/z 249 and m/z 207 typical to that reported for macrocarpals¹⁶; they were annotated as macrocarpals isomers. In addition, ten signals were also detected and identified as euglobals through their intense peak at m/z 249 and less intense peak at m/z 207 as previously reported^{16,30}. Moreover, four dimeric phloroglucinols were identified as sideroxylonal A/B/C and grandinal. They were characterized by the presence of molecular ion peak at m/z 499 and daughter ions at m/z 471, m/z 249 and m/z 207. All these metabolites were previously reported in *E. sideroxylon* leaves and flowers^{9,16}. Other phloroglucinols, formylated phloroglucinol compounds (FPC 1–10), were tentatively characterized by comparing their masses with the previously reported data^{30,32}.

Nine gallic and ellagic acid derivatives were detected. Fragments of galloyl moiety (m/z 169) and product ions due to its loss $[M-H-169]^-$ were detected in the mass chromatogram of the corresponding peaks.

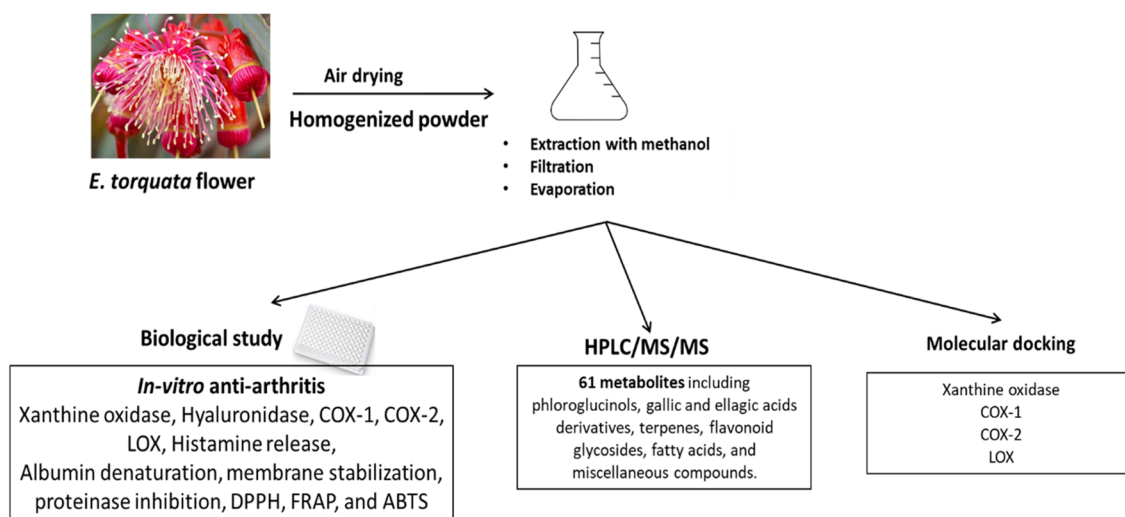


Figure 1. Scheme for extraction, biological evaluation, and chemical analysis of *E. torquata* flowers (ETME).

Assay	ETME IC ₅₀ /EC ₅₀ (μg/mL)	Reference drugs						
		Ascorbic acid	Celecoxib	Indomethacin	Diclofenac	Sodium aurothiomalate	Kojic acid	Oxypurinol
DPPH	5.8 ± 0.69	2.2 ± 1.6	–	–	–	–	–	–
FRAP	7.4 ± 0.96	3.9 ± 1.9	–	–	–	–	–	–
ABTS	40.3 ± 1.2	21.4 ± 1.6	–	–	–	–	–	–
COX-1	267.02 ± 1.3	–	248.9 ± 0.78	–	–	–	–	–
COX-2	0.42 ± 0.82	–	0.26 ± 0.99	–	–	–	–	–
SI	635	–	935	–	–	–	–	–
LOX	48.4 ± 1.4	–	–	23.4 ± 0.83	–	–	–	–
Albumin denaturation	26.2 ± 0.76	–	–	–	15.12 ± 1.8	–	–	–
Membrane stabilization	24.4 ± 1.6	–	–	–	17.02 ± 1.3	–	–	–
Protease	31.1 ± 0.86	–	–	–	25.14 ± 1.2	–	–	–
Histamine release	33.2 ± 1.4	–	–	–	17.94 ± 1.4	–	–	–
Hyaluronidase	31.06 ± 0.58	–	–	–	–	20.03 ± 0.96	–	–
Tyrosinase	60.8 ± 1.7	–	–	–	–	–	52.0 ± 0.98	–
Xanthine oxidase	22.2 ± 1.3	–	–	–	–	–	–	14.7 ± 0.93

Table 1. In vitro antioxidant, anti-inflammatory and antiarthritic potentials of *E. torquata* flower methanol extract (ETME). All experiments were carried out in a triplicate manner and values are expressed as mean ± SD. SI is COX selectivity index calculated as IC₅₀ (COX-1)/IC₅₀ (COX-2).

Regarding terpenes, the presence of molecular ion peak at m/z 455 and daughter ions at m/z 249 and m/z 207 allowed the tentative identification of ursolic/betulonic acid. The $[M-H]^-$ m/z 471 exceeds that of ursolic/betulonic acid by 16 Da, which means extra hydroxyl group, this leads to its identification as hydroxy ursolic acid/hederagenin.

Five flavonoid glycosides; kaempferol, quercetin, and isorhamnetin derivatives have been tentatively identified through their fragmentation pattern that showed the presence of m/z 285, 301, and 315, respectively. This fragmentation pattern showed the loss of rhamnose (m/z 146), glucose (m/z 162), rutinoid (m/z 308), and gallate (m/z 152) fragments that appeared independently to be cleaved from the main structure.

Two fatty acids were tentatively identified. Their fragmentation is consistent with the previously reported data¹⁶. The two fatty acids are hydroxy tetracosanoic acid and hydroxy octadecadienoic acid that showed molecular ion peaks at m/z 383 and m/z 295, respectively.

Two miscellaneous compounds; one oleuropic acid derivative, eucalmaidin C, and one phenolic compound, glucosyl-dihydroxy-isobutylchromone, were also identified in ETME.

Virtual screening and drug likeness analysis

We have previously studied the binding affinity of several organic acids, phenolics and flavonoids towards some of the prominent pro-inflammatory enzyme targets by the aid of the molecular docking computational tool^{25,27}. Herein, we docked 35 phloroglucinols, the most abundant identified class of compounds, to COX-1, COX-2, 5-LOX, and XO Table 3, so that we could virtually investigate their binding affinity and blocking potential towards these key enzymes mediating the inflammation process. This would help identify the key compounds, which could contribute to the extracts' inhibitory potential against these target proteins and present them as leads for developing novel drug candidates of natural origin. Regarding COX-1 and COX-2 enzymes, it was observed that the docked phloroglucinols showed generally a very good binding affinity to both target enzymes, however they showed much better affinity towards COX-2, which confirms their COX-2/COX-1 selectivity as revealed in the in vitro assays. Sideroxylonal C showed the best affinity (minimum docking score) to COX-1 with a docking score of – 14.23 kcal/mol. However, macrocarpal I showed the highest affinity towards COX-2 with a docking score of – 7.4 kcal/mol and retained a score of – 10.99 kcal/mole towards COX-1, which indicates high selectivity towards COX-2. Docking to 5-LOX revealed appreciable binding affinity of the docked phloroglucinols at the binding site of the target enzyme reflected by the docking score values that ranged from – 18.00 to – 10.67 kcal/mol. The best binding affinity was shown by sideroxylonal B. As for XO, the binding affinity showed by the phloroglucinol derivatives docked into this target enzyme was not as good as it was in the other three targets, as the docking score ranged only from – 7.87 to – 6.03 kcal/mol. Sideroxylonal B was the best docked compound with the least minimum docking score of – 7.87 kcal/mol. It afforded two hydrogen bonding interactions with Ser876 and Val1011, neither of which was reported by the co-crystallized inhibitor quercetin. In view of these results, we could conclude that the extracts' compounds are most likely targeting COX/LOX enzymatic pathway.

We also investigated the drug likeness potential of the phloroglucinol derivatives identified in the extract by measuring the descriptors associated with drug likeness and oral bioavailability, namely the molecular weight, number of H-bond acceptors (lip_acc), number of H-bond donors (lip_don), and the partition coefficient

Identification	[M-H] ⁻	Main fragments	References
Phloroglucinols			
Grandinol	251	249, 207	28
Jensenone	265	250, 193, 165	6
Euglobal T1, IIb, IIc, or Ia1	385	249, 207	29
Euglobal G1-10 isomers	385	249, 207	29
Euglobal In3, Iva, III, V, or VII	453	385, 249, 207	29
Macrocarpal A, B, H, K, E or D	471	249, 207	16
Macrocarpal AM1/N	485	207	16
Macrocarpal I/J	489	471, 265, 249	16
Sideroxylonal A, B, or C	499	471, 249, 207	9
Grandinal	499	249, 207	9
FPC (1)	925	893, 497, 249	30
FPC (2)	653	471, 249, 207	
FPC (3)	635	489, 249, 207	
FPC (4)	403	375, 249, 207	
FPC (5)	907	471, 249, 207	
FPC (6)	721	471, 385, 249	
FPC (7)	635	453, 249, 207	
FPC (8)	703	453, 249, 207	
FPC (9)	857	453, 249, 207	
FPC (10)	607	453, 250, 207	
Gallic and Ellagic acids derivatives			
Methyl gallate	183	183, 169, 125	31
Ellagic acid	301	301, 257, 229	31
Methylellagic acid	315	315, 301, 169	31
Methyldigallic acid	335	183, 169, 124	31
Methyldigallic acid gallate	487	335, 183, 169	31
Tetra- <i>O</i> -galloyl-glucose	787	635, 465, 169	15
Digalloyl- <i>O</i> -methyl-glucose	497	313, 271, 169	15
Trigalloylglucose	635	477, 301, 169	15
Galloyl glucose	331	316, 169	15
Terpenes			
Nor triterpene	453	385, 249	16
Eucalyptanoic acid	453	385, 207	16
Ursolic/betulinic acid	455	369, 249, 207	16
Hydroxy ursolic acid/Hederagenin	471	385, 249	16
Eucalyptic/eucalyptolic acid	647	471, 249, 207	16
Lactone of asiatic acid	485	457, 439, 425	16
Arjunolic acid	487	458, 249, 207	16
Globulusin A	483	425, 249, 165	32
Flavonoid glycosides			
Kaempferol-rhamnosylrutinoside	739	595, 285, 237	33
Quercetin <i>O</i> -rhamnosylrutinoside	755	593, 301, 279	34
Quercetin- <i>O</i> -glucoside-gallate	615	463, 301	31
Isorhamnetin <i>O</i> -rhamnosylrutinoside	769	623, 315, 271	33
Isorhamnetin <i>O</i> -diglucoside	639	315, 301	31
Fatty acids			
Hydroxy tetracosanoic acid	383	365, 325, 299	16
Hydroxy octadecadienoic acid C18:2	295	277, 171, 113	16
Miscellaneous			
Eucalmaindin C	525	463, 239, 197	35
Glucosyl-dihydroxy-isobutylchromone	395	233, 275, 189	35

Table 2. Secondary metabolites identified in *E. torquata* flower using HPLC–PDA–MS.

Compound name	Function score (Kcal/mole)			XO
	5-LOX	COX-1	COX-2	
Macrocarpal I	– 14.55	– 10.99	– 17.40	– 7.33
Sideroxylonal B	– 18.00	– 11.09	– 17.32	– 7.87
Sideroxylonal C	– 15.33	– 14.23	– 16.62	– 7.02
Macrocarpal J	– 13.31	– 12.46	– 16.28	– 6.94
Euglobal G7	– 10.81	– 9.70	– 15.37	– 6.49
Macrocarpal K	– 13.71	– 12.31	– 15.24	– 7.55
Macrocarpal H	– 14.43	– 10.43	– 15.13	– 6.08
Macrocarpal N	– 13.32	– 12.34	– 15.04	– 6.66
Euglobal Ia1	– 12.42	– 9.24	– 14.91	– 6.89
Macrocarpal L	– 13.19	– 10.67	– 14.74	– 7.00
Grandinal	– 15.16	– 13.92	– 14.59	– 7.40
Macrocarpal D	– 12.80	– 7.43	– 14.29	– 6.43
Euglobal III	– 12.70	– 11.22	– 13.92	– 6.03
Euglobal IIC	– 10.08	– 9.96	– 13.86	– 6.79
Euglobal V	– 10.93	– 12.44	– 13.80	– 6.49
Macrocarpal A	– 16.14	– 10.45	– 13.29	– 6.76
Eucalyptal E	– 15.50	– 10.16	– 13.27	– 7.02
Euglobal G3	– 12.05	– 9.30	– 13.19	– 7.30
Euglobal G6	– 12.00	– 10.68	– 13.10	– 7.41
Macrocarpal K	– 14.38	– 11.14	– 13.01	– 7.14
Euglobal IIb	– 12.97	– 10.79	– 12.80	– 7.59
Jensenone	– 11.42	– 8.85	– 12.75	– 7.03
Sideroxylonal A	– 17.42	– 12.29	– 12.55	– 7.06
Euglobal Iva	– 13.02	– 9.16	– 12.51	– 7.18
Euglobal In-3	– 12.01	– 11.60	– 12.49	– 6.57
Euglobal G2	– 11.91	– 11.58	– 12.31	– 6.88
Euglobal G9	– 11.23	– 9.04	– 12.29	– 7.47
Macrocarpal B	– 14.25	– 10.39	– 11.88	– 6.59
Euglobal G8	– 11.96	– 10.66	– 11.63	– 6.94
Euglobal G4	– 11.37	– 8.94	– 10.85	– 7.11
Euglobal T1	– 11.53	Failed	– 10.80	– 7.18
Grandinol	– 10.67	– 9.69	– 10.38	– 6.33
Euglobal VII	– 10.97	– 9.39	– 10.36	– 7.70
Euglobal G1	– 11.73	Failed	– 10.04	– 7.12
Euglobal G10	– 11.58	– 7.46	– 9.42	– 7.33

Table 3. Docking scores of the identified phloroglucinols from *E. torquata* flowers.

(logP(o/w)). Among the investigated compounds, only 8 showed to have drug likeness potential as they obeyed Lipiniski's rule of five, the most common rule used by medicinal chemists to check the oral bioavailability of newly developed drug candidates, Table 4. Interestingly, these compounds included sideroxylonal C and macrocarpal I, which showed the best binding affinity towards COX-1 and COX-2 enzymes, respectively and sideroxylonal B as well that was the best docked compound into both 5-LOX and XO enzymes. These three compounds, thus, can be very promising lead hits for developing novel anti-inflammatory agents of natural origin.

Compound name	Weight	lip_acc	lip_don	logP(o/w)	lip_druglike	lip_violation
Macrocarpal I	490.64	7	5	5.76	1	0
Sideroxylonal B/C	500.50	10	5	5.03	1	1
Euglobal G7	386.49	5	2	4.37	1	0
Macrocarpal K/H	472.62	6	4	5.81	1	1
Macrocarpal N	486.60	7	3	4.69	1	1

Table 4. Drug likeness analysis of phloroglucinol derivatives identified in *E. torquata*.

Discussion

Several studies have been conducted to discover plant-derived natural drugs. We herein report the effective role of *E. torquata* flowers in the treatment of gouty arthritis. A close relationship has been highlighted between uric acid and oxidative stress, where a large amount of reactive oxygen species is produced along with uric acid production. Antioxidants are considered of great importance in the management of hyperuricemia³⁶. Also, oxidative stress plays a key role in the pathogenesis of gout and is responsible for a series of inflammatory pathways^{37,38}.

Comparable antioxidant properties were reported for several *Eucalyptus* species; *E. camaldulensis*^{39,40}, *E. globulus* leaves and bark^{41,42} and *E. sideroxylon* bark⁴³. In addition, several Myrtaceae plants are rich in polyphenolics such as *Eugenia uniflora*, *Syzygium samarangense*, *Syzygium jambos*, *Syzygium aqueum*^{44–47}, and *Callistemon citrinus*⁴⁸.

It is well documented that pro-inflammatory enzymes play an essential role in inflammation pathogenesis through different pathways. So, cyclooxygenases and lipoxygenases inhibition are considered as targets for the management of oxidative stress associated diseases and inflammation that contribute in the amelioration of gouty inflammation²⁴. It was reported that the selective COX-2 inhibitors that showed 2–100 fold difference in the concentrations needed to inhibit COX-2 versus COX-1, decreases prostaglandins levels at the inflammatory sites only and has no effect on gastric mucosal prostaglandin levels⁴⁹. Our results are in accordance with that reported about the inhibition potential of several *Eucalyptus* species oils and extracts against cyclooxygenases and lipoxygenases enzymes^{50–52}.

Denaturation of tissue proteins is well documented in the pathogenesis of inflammatory events like arthritis. The protection against protein denaturation is one of NSAIDs mechanisms of action. In addition, red blood cell hemolysis inhibition may provide insights into the process of inflammation. Stabilization of the red blood cell membranes may delay or prevent the lysis and the consequent cytoplasmic contents release and the inflammatory response. So, plants that can interfere with these responses could be very useful for developing novel anti-arthritic drugs⁵³. Natural extracts from other *Eucalyptus* species produced similar albumin denaturation and cell membrane stabilization activities including *E. obliqua* leaves⁵⁴, *E. sideroxylon* bark⁹ and *E. globulus* leaves⁵⁵.

Moreover, during inflammation, leukocytes release lysosomal enzymes, including proteases, triggering further tissue damage and consequent inflammation. Proteinases have been related to arthritic reactions, where a significant level of protection was provided by inhibitors of proteinase enzyme. The observed protease inhibitory potential of ETME is in accordance with the reported data about the proteinase inhibitory activity of other *Eucalyptus* species^{9,56}.

Regarding histamine, it is a very important mediator in inflammatory conditions. The synovial fluids from patients with acute gout have significantly high levels of histamine⁵⁷. In addition, hyaluronidase enhances mast cells degranulation and releases inflammatory mediators leading to several pathological conditions including arthritis²⁴. Tyrosinase enzyme, on the other hand, is a multifunctional, widely distributed enzyme in nature, which when accrued in excess, melanin results in hyperpigmentation disorders, Parkinson's disease, and others oxidative stress disorders like arthritis⁵⁸. Interestingly, *E. globulus* extract was previously reported to inhibit histamine release, hyaluronidase and tyrosinase enzymes, which comes in agreement of our data in this study^{59–61}.

The oxidation xanthine and hypoxanthine to uric acid is catalyzed by xanthine oxidase⁶². Hyperuricemia is caused by the over expression of xanthine oxidase resulting in high levels of uric acid that contribute to the deposition of monosodium urate in the joint cavity, which in turn activates inflammatory cytokines, leading to gouty arthritis. Therefore, keeping xanthine oxidase and serum uric acid levels within normal range is very important in treating or ameliorating this distressfully agonizing condition⁶³. Noteworthy, *Eucalyptus deglupta* was reported to inhibit xanthine oxidase⁶⁴, which, along with our results, confirm the value of this genus in managing inflammation and gouty arthritis.

The secondary metabolites identified in ETME are responsible for its newly explored anti-hyperuricemic and anti-inflammatory potentials. Ursolic acid, for instance, has reported potent hypouricemic activity and XO inhibitory activities⁶⁵. In addition, several studies have reported flavonoids as potent inhibitors of COX, 5-LOX and XO enzymes^{66–68}. Furthermore, methyl gallate was reported to possess not only xanthine oxidase inhibitory activity but it also suppressed superoxide anion generated by XO⁶⁹ in addition to its potent COX-I, COX-II and 5-LOX inhibitory activities^{70,71}. Ellagic acid was also reported to strongly inhibit XO⁷² and scavenge DPPH stable radical⁷³. Phloroglucinols and their derivatives have been reported for their anti-inflammatory and antioxidant activities^{74–76}.

The extensively explored *E. torquata* flowers potential, herein, for the management of gouty arthritis suggests its future deep investigation at in vivo and clinical levels for the discovery of novel gouty arthritis drugs. Further chemical investigation on the plant to isolate its bioactive constituents and drug development is in demand as well.

Conclusion

Eucalyptus torquata flowers extract showed potent in vitro anti-gouty activity with remarkable selective COX-2 inhibitory potential. Several secondary metabolites belonging to different phytochemical classes were identified in the extract. The most abundant was the phloroglucinols. In view of our in-silico results, some of the identified metabolites from *E. torquata* flowers can be considered as novel leads for the discovery and development of drug candidates of natural origin that could be used to manage and combat gouty arthritis.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 22 February 2023; Accepted: 20 October 2023

Published online: 31 October 2023

References

- Im, K. H. *et al.* Antioxidant, anti-melanogenic and anti-wrinkle effects of *Phellinus vaninii*. *Mycobiology* **47**(4), 494–505 (2019).
- Luo, Y. *et al.* Metabolic profiling of human plasma reveals the activation of 5-lipoxygenase in the acute attack of gouty arthritis. *Rheumatology* **58**(2), 345–351 (2019).
- Lin, K.-C., Lin, H. Y. & Chou, P. The interaction between uric acid level and other risk factors on the development of gout among asymptomatic hyperuricemic men in a prospective study. *J. Rheumat.* **27**(6), 1501–1505 (2000).
- Janssens, H. J. *et al.* Use of oral prednisolone or naproxen for the treatment of gout arthritis: A double-blind, randomised equivalence trial. *The Lancet* **371**(9627), 1854–1860 (2008).
- Mohsen, A. M. *et al.* Thymol-Loaded Eudragit RS30D cationic nanoparticles-based hydrogels for topical application in wounds: In vitro and in vivo evaluation. *Pharmaceutics* **15**(1), 19 (2023).
- Okba, M. M. *et al.* HPLC-PDA-ESI-MS/MS profiling and anti-biofilm potential of eucalyptus sideroxylon flowers. *Antibiotics* **10**(7), 761 (2021).
- Abdel-Baki, P. M. *et al.* Irigenin, a novel lead from *Iris confusa* for management of *Helicobacter pylori* infection with selective COX-2 and Hp IMPDH inhibitory potential. *Sci. Rep.* **12**(1), 11457 (2022).
- Okba, M. M. *et al.* UPLC-ESI-MS/MS profiling of the underground parts of common *Iris* species in relation to their anti-virulence activities against *Staphylococcus aureus*. *J. Ethnopharmacol.* **282**, 114658 (2022).
- Ashour, R. M. *et al.* Eucalyptus sideroxylon bark anti-inflammatory potential, its UPLC-PDA-ESI-qTOF-MS profiling, and isolation of a new phloroglucinol. *J. Chromatogr. Sci.* **57**(6), 565–574 (2019).
- Ezzat, S. M. *et al.* The hidden mechanism beyond ginger (*Zingiber officinale* Rosc.) potent in vivo and in vitro anti-inflammatory activity. *J. Ethnopharmacol.* **214**, 113–123 (2018).
- He, Y. *et al.* Curcumin, inflammation, and chronic diseases: How are they linked?. *Molecules* **20**(5), 9183–9213 (2015).
- Perera, H. D. *et al.* In vitro pro-inflammatory enzyme inhibition and anti-oxidant potential of selected Sri Lankan medicinal plants. *BMC Complement. Altern. Med.* **18**(1), 1–15 (2018).
- Chen, C. H. *et al.* Antioxidant activity of some plant extracts towards xanthine oxidase, lipoxygenase and tyrosinase. *Molecules* **14**(8), 2947–2958 (2009).
- Branco, A. C. C. *et al.* Role of histamine in modulating the immune response and inflammation. *Mediat. Inflamm.* **2018**, 896 (2018).
- Ghareeb, M. A. *et al.* Chemical profiling of polyphenolics in *Eucalyptus globulus* and evaluation of its hepato–renal protective potential against cyclophosphamide induced toxicity in mice. *Antioxidants* **8**(9), 415 (2019).
- Okba, M. M., El Gedaily, R. A. & Ashour, R. M. UPLC–PDA–ESI–qTOF-MS profiling and potent anti-HSV-II activity of *Eucalyptus sideroxylon* leaves. *J. Chromatogr. B* **1068**, 335–342 (2017).
- Salehi, B. *et al.* Insights into *Eucalyptus* genus chemical constituents, biological activities and health-promoting effects. *Trends Food Sci. Technol.* **91**, 609–624 (2019).
- Ashour, H. M. Antibacterial, antifungal, and anticancer activities of volatile oils and extracts from stems, leaves, and flowers of *Eucalyptus sideroxylon* and *Eucalyptus torquata*. *Cancer Biol. Therapy* **7**(3), 399–403 (2008).
- Dudonne, S. *et al.* Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *J. Agric. Food Chem.* **57**(5), 1768–1774 (2009).
- Kumar, A. N. *et al.* Antioxidant, cytoprotective and anti-inflammatory activities of stem bark extract of *Semecarpus anacardium*. *Asian J. Pharm. Clin. Res.* **6**(1), 213–219 (2013).
- Shilpa, K. *et al.* Investigation of anti-arthritis activity (in-vitro models) of *Hibiscus hispidissimus* Griffith. *J. Phytopharmacol.* **7**(1), 60–65 (2018).
- Lima, R. D. C. L. *et al.* Effects of extracts of leaves from *Sparattosperma leucanthum* on hyperuricemia and gouty arthritis. *J. Ethnopharmacol.* **161**, 194–199 (2015).
- Di Petrillo, A. *et al.* Tyrosinase inhibition and antioxidant properties of *Asphodelus microcarpus* extracts. *BMC Complement. Altern. Med.* **16**(1), 1–9 (2016).
- Perera, H. D. S. M. *et al.* In vitro pro-inflammatory enzyme inhibition and anti-oxidant potential of selected Sri Lankan medicinal plants. *BMC Complement. Altern. Med.* **18**(1), 271 (2018).
- Sobeh, M. *et al.* *Albizia anthelmintica*: HPLC-MS/MS profiling and in vivo anti-inflammatory, pain killing and antipyretic activities of its leaf extract. *Biomed. Pharmacother.* **115**, 108882 (2019).
- El-Hawary, S. S. *et al.* HPLC-PDA-MS/MS profiling of secondary metabolites from *Opuntia ficus-indica* cladode, peel and fruit pulp extracts and their antioxidant, neuroprotective effect in rats with aluminum chloride induced neurotoxicity. *Saudi J. Biol. Sci.* **27**(10), 2829–2838 (2020).
- Sobeh, M. *et al.* *Thymus algeriensis* and *Thymus fontanesii*: Chemical composition, in vivo antiinflammatory, pain killing and antipyretic activities: A comprehensive comparison. *Biomolecules* **10**(4), 599 (2020).
- Ossipov, V. *et al.* UPLC-PDA-Q Exactive Orbitrap-MS profiling of the lipophilic compounds product isolated from *Eucalyptus viminalis* plants. *Heliyon* **6**, 12 (2020).
- Singh, I. P. *et al.* Phloroglucinol compounds of natural origin: Synthetic aspects. *Nat. Prod. Rep.* **27**(3), 393–416 (2010).
- Eyles, A., Davies, N. W. & Mohammed, C. Novel detection of formulated phloroglucinol compounds (FPC s) in the wound wood of *Eucalyptus globulus* and *E. nitens*. *J. Chem. Ecol.* **29**, 881–898 (2003).
- González-Burgos, E. *et al.* Antioxidant activity, neuroprotective properties and bioactive constituents analysis of varying polarity extracts from *Eucalyptus globulus* leaves. *J. Food Drug Anal.* **26**(4), 1293–1302 (2018).
- Hasegawa, T. *et al.* Bioactive monoterpene glycosides conjugated with gallic acid from the leaves of *Eucalyptus globulus*. *Phytochemistry* **69**(3), 747–753 (2008).
- Jang, G. H. *et al.* Characterization and quantification of flavonoid glycosides in the *Prunus* genus by UPLC-DAD-QTOF/MS. *Saudi J. Biol. Sci.* **25**(8), 1622–1631 (2018).
- Farias, L. D. S. & Mendez, A. S. LC/ESI-MS method applied to characterization of flavonoids glycosides in *B. forficata* subsp. *pruinosa*. *Quim. Nova* **37**, 483–486 (2014).
- Tian, L.-W. *et al.* Eucalmaidins A– E,(+)–oleuropeic acid derivatives from the fresh leaves of *Eucalyptus maideni*. *J. Nat. Prod.* **72**(9), 1608–1611 (2009).
- Li, L. *et al.* Anti-gouty arthritis and antihyperuricemia effects of sunflower (*Helianthus annuus*) head extract in gouty and hyperuricemia animal models. *BioMed. Res. Int.* **2017**, 852 (2017).
- Umamaheswari, M. *et al.* Xanthine oxidase inhibitory activity of some Indian medical plants. *J. Ethnopharmacol.* **109**(3), 547–551 (2007).
- Li, P. *et al.* Palmitic acid and β -hydroxybutyrate induce inflammatory responses in bovine endometrial cells by activating oxidative stress-mediated NF- κ B signaling. *Molecules* **24**(13), 2421 (2019).
- El-Ghorab, A. H. *et al.* Antioxidant activity of Egyptian *Eucalyptus camaldulensis* var. *brevirostris* leaf extracts. *Food/Nahrung* **47**(1), 41–45 (2003).

40. Nwabor, O. F., Vongkamjan, K. & Voravuthikunchai, S. P. Antioxidant properties and antibacterial effects of *Eucalyptus camaldulensis* ethanolic leaf extract on biofilm formation, motility, hemolysin production, and cell membrane of the foodborne pathogen *Listeria monocytogenes*. *Foodborne Pathogens Dis.* **16**(8), 581–589 (2019).
41. Remini, H. *et al.* Phytochemical analysis and antioxidant activity of *Eucalyptus globulus*: A comparative study between fruits and leaves extracts. *J. Chem. Eng. Bioanal. Chem.* **1**, 23–29 (2016).
42. Vázquez, G. *et al.* Antioxidant activity and phenolic content of chestnut (*Castanea sativa*) shell and eucalyptus (*Eucalyptus globulus*) bark extracts. *Ind. Crops Prod.* **28**(3), 279–285 (2008).
43. Miranda, I. *et al.* The bark of *Eucalyptus sideroxylon* as a source of phenolic extracts with anti-oxidant properties. *Ind. Crops Prod.* **82**, 81–87 (2016).
44. Sobeh, M. *et al.* Phenolic compounds from *Syzygium jambos* (Myrtaceae) exhibit distinct antioxidant and hepatoprotective activities in vivo. *J. Funct. Foods* **41**, 223–231 (2018).
45. Sobeh, M. *et al.* Chemical profiling of secondary metabolites of *Eugenia uniflora* and their antioxidant, anti-inflammatory, pain killing and anti-diabetic activities: A comprehensive approach. *J. Ethnopharmacol.* **240**, 111939 (2019).
46. Sobeh, M. *et al.* *Syzygium aqueum*: A polyphenol-rich leaf extract exhibits antioxidant, hepatoprotective, pain-killing and anti-inflammatory activities in animal models. *Front. Pharmacol.* **9**, 566 (2018).
47. Sobeh, M. *et al.* High resolution UPLC-MS/MS profiling of polyphenolics in the methanol extract of *Syzygium samarangense* leaves and its hepatoprotective activity in rats with CCl₄-induced hepatic damage. *Food Chem. Toxicol.* **113**, 145–153 (2018).
48. Shehabeldine, A. M. *et al.* Callistemon citrinus bioactive metabolites as new inhibitors of methicillin-resistant *Staphylococcus aureus* biofilm formation. *J. Ethnopharmacol.* **254**, 112669 (2020).
49. Everts, B., Währborg, P. & Hedner, T. COX-2-Specific inhibitors—the emergence of a new class of analgesic and anti-inflammatory drugs. *Clin. Rheumatol.* **19**(5), 331–343 (2000).
50. Tiwari, A. *et al.* *Eucalyptus robusta* leaves methanolic extract suppresses inflammatory mediators by specifically targeting TLR4/TLR9, MPO, COX2, iNOS and inflammatory cytokines in experimentally-induced endometritis in rats. *J. Ethnopharmacol.* **213**, 149–158 (2018).
51. Mondal, M. *et al.* Analgesic and anti-inflammatory potential of essential oil of *Eucalyptus camaldulensis* leaf: In vivo and in silico studies. *Nat. Prod. Commun.* **16**(4), 1934 (2021).
52. Göger, G. *et al.* In vitro antimicrobial, antioxidant and anti-inflammatory evaluation of *Eucalyptus globulus* essential oil. *Nat. Volat. Essential Oils* **7**(3), 1–11 (2020).
53. El-Shiekh, R. A. *et al.* A mechanistic study of *Solenostemma argel* as anti-rheumatic agent in relation to its metabolite profile using UPLC/HRMS. *J. Ethnopharmacol.* **265**, 113341 (2021).
54. Sabiu, S. & Ashafa, A. Membrane stabilization and kinetics of carbohydrate metabolizing enzymes (α -amylase and α -glucosidase) inhibitory potentials of *Eucalyptus obliqua* L. Her. (Myrtaceae) Blakely ethanolic leaf extract: An in vitro assessment. *South Afr. J. Bot.* **105**, 264–269 (2016).
55. Sharma, A. D. *et al.* Phytochemicals, in-vitro antioxidant, in-vitro antibacterial and anti-inflammatory activity of methanolic leaf extract from *Eucalyptus globulus*. *Life Sci. J.* **17**(11), 896 (2020).
56. Khoury, J. *et al.* Evidence for in vitro antiprophidial properties of aqueous buds extract of *Eucalyptus* against *Montivipera bornmueleri* venom. *J. Venom Res.* **8**, 25 (2017).
57. Reber, L. L. *et al.* Contribution of mast cell-derived interleukin-1 β to uric acid crystal-induced acute arthritis in mice. *Arthritis Rheumatol.* **66**(10), 2881–2891 (2014).
58. Morais, D. V. D. *et al.* Antioxidant, photoprotective and inhibitory activity of tyrosinase in extracts of *Dalbergia ecastaphyllum*. *PLoS One* **13**(11), e0207510 (2018).
59. Ikawati, Z., Wahyuono, S. & Maeyama, K. Screening of several Indonesian medicinal plants for their inhibitory effect on histamine release from RBL-2H3 cells. *J. Ethnopharmacol.* **75**(2–3), 249–256 (2001).
60. Nile, S. H. & Keum, Y. S. Chemical composition, antioxidant, anti-inflammatory and antitumor activities of *Eucalyptus globulus* Labill. *Indian J. Exp. Biol.* **56**, 734–742 (2018).
61. Sugimoto, K. *et al.* Hydrolyzable tannins as antioxidants in the leaf extract of *Eucalyptus globulus* possessing tyrosinase and hyaluronidase inhibitory activities. *Food Sci. Technol. Res.* **15**(3), 331–336 (2009).
62. Kong, L. *et al.* Inhibition of xanthine oxidase by some Chinese medicinal plants used to treat gout. *J. Ethnopharmacol.* **73**(1–2), 199–207 (2000).
63. Ouyang, H. *et al.* Antioxidant and xanthine oxidase inhibitory activities of total polyphenols from onion. *Saudi J. Biol. Sci.* **25**(7), 1509–1513 (2018).
64. Ansari, K. A. *et al.* Xanthine Oxidase Inhibition by Some Medicinal Plants. Portal Regional da BVS Informação e Conhecimento para a Saúde pesquisa.bvsalud.org (2011).
65. Abu-Gharbieh, E. *et al.* Antihyperuricemic and xanthine oxidase inhibitory activities of *Tribulus arabicus* and its isolated compound, ursolic acid: In vitro and in vivo investigation and docking simulations. *PLoS One* **13**(8), e0202572 (2018).
66. Harborne, J. B. & Williams, C. A. Advances in flavonoid research since 1992. *Phytochemistry* **55**(6), 481–504 (2000).
67. Cos, P. *et al.* Structure—activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *J. Nat. Prod.* **61**(1), 71–76 (1998).
68. Lin, S. *et al.* Dietary flavonoids as xanthine oxidase inhibitors: Structure—affinity and structure—activity relationships. *J. Agric. Food Chem.* **63**(35), 7784–7794 (2015).
69. Masuoka, N., Nihei, K. I. & Kubo, I. Xanthine oxidase inhibitory activity of alkyl gallates. *Mol. Nutr. Food Res.* **50**(8), 725–731 (2006).
70. Kim, S. J. *et al.* Effects of methyl gallate on arachidonic acid metabolizing enzymes: Cyclooxygenase-2 and 5-lipoxygenase in mouse bone marrow-derived mast cells. *Arch. Pharmacol. Res.* **29**(10), 874–878 (2006).
71. Kamatham, S., Kumar, N. & Gudipalli, P. Isolation and characterization of gallic acid and methyl gallate from the seed coats of *Givotia rotleriformis* Griff. and their anti-proliferative effect on human epidermoid carcinoma A431 cells. *Toxicol. Rep.* **2**, 520–529 (2015).
72. Sato, V. H. *et al.* Pharmacological effects of Chatuphalatika in hyperuricemia of gout. *Pharmaceut. Biol.* **56**(1), 76–85 (2018).
73. Amakura, Y. *et al.* Constituents and their antioxidative effects in eucalyptus leaf extract used as a natural food additive. *Food Chem.* **77**(1), 47–56 (2002).
74. Li, Y. *et al.* The immunoregulation of Th17 in host against intracellular bacterial infection. *Mediat. Inflamm.* **2018**, 13 (2018).
75. Singh, I. P. *et al.* Phloroglucinol compounds of therapeutic interest: Global patent and technology status. *Expert Opin. Therapeut. Patents* **19**(6), 847–866 (2009).
76. Crockett, S. L. *et al.* Anti-inflammatory phloroglucinol derivatives from *Hypericum empetrifolium*. *Phytochem. Lett.* **1**(1), 37–43 (2008).

Author contributions

R.M.S.A., R.A.E., M.S., M.M.O.: writing—original draft, data curation, formal analysis, funding acquisition, investigation, resources, methodology. R.M.S.A., R.A.E., M.S.: conceptualization, supervision, writing—review & editing. R.M.S.A., R.A.E., M.S., M.A.O.A., M.M.A., M.M.O.: investigation, methodology, resources.

Funding

Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-023-45499-0>.

Correspondence and requests for materials should be addressed to M.M.O.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2023