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Crinum jagus: antiproliferative studies of extracts on HepG2 cell line and in silico assessment of phytoconstituents as potential inhibitors of p53–mortalin interaction

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Abstract

Background Hepatocellular carcinoma (HCC) is the third leading cause of cancer death worldwide and has a poor prognosis in black Africans. Traditional herbal practitioners in southwestern Nigeria use *Crinum jagus* (J. Thompson) Dandy for cancer treatment. This study screens methanol and ethyl acetate extracts of *C. jagus* leaves for activity against hepatocellular carcinoma (HepG2) cell line using the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay. The antiproliferative properties of the extracts were assessed by comparing their IC_{50} values with that of the standard drug, cisplatin.The GC–MS technique was used to identify the phytoconstituents in the extracts. The drug-likeness of each identified phytoconstituents in the extracts was determined by following Lipinski's rule of five. In addition, phytoconstituents having drug-like properties were screened as potential inhibitors of the p53–mortalin interaction by docking them against the mortalin residues 3N8E and 4KBO using Swissdock.

Results For the antiproliferative study, the IC_{50} values obtained for cisplatin, methanol, and ethyl acetate extracts of leaves were 5 µg/mL, 5 µg/mL, and 70 µg/mL, respectively, indicating that the methanol extract and cisplatin possess comparable antiproliferative properties. Hexadecanoic acid, hexadecanoic acid methyl ester, tangeretin, galanthamine, and crinamine, which were part of the constituents identified in the leaves, possess drug-like properties and are known to show cytotoxic properties against several cancer cell lines. On docking with mortalin residue 3N8E, hexadecanoic acid and hexadecanoic acid methyl ester had comparable binding energy (− 8.21 kcal mol^{-1}) with withaferin A and withanone (8.29 kcal mol^{-1} and 8.14 kcal mol^{-1}). Hexadecanoic acid, hexadecanoic acid methyl ester, and galanthamine had binding energy of − 7.66, − 7.45, and − 7.47 kcal mol^{-1}, respectively, with mortalin residue, 4KBO, comparable to values of − 7.68 and − 7.59 kcal mol−1 obtained for withaferin A and withanone, respectively.

Conclusion The methanol extract of *C. jagus* leaves demonstrated remarkable antiproliferative activities against HepG2, justifying its use in traditional medicine for cancer treatment. The ethyl acetate and methanol extracts contain drug-like compounds with known cytotoxic properties against several cancer cell lines. Some of these compounds (hexadecanoic acid, hexadecanoic acid methyl ester, tangeretin, and galanthamine) are inhibitors of the p53–mortalin interaction.

Keywords Antiproliferative, Mortalin, Docking, MTT, Cancer, GCMS, HepG2, Druglikeness, Phytochemicals

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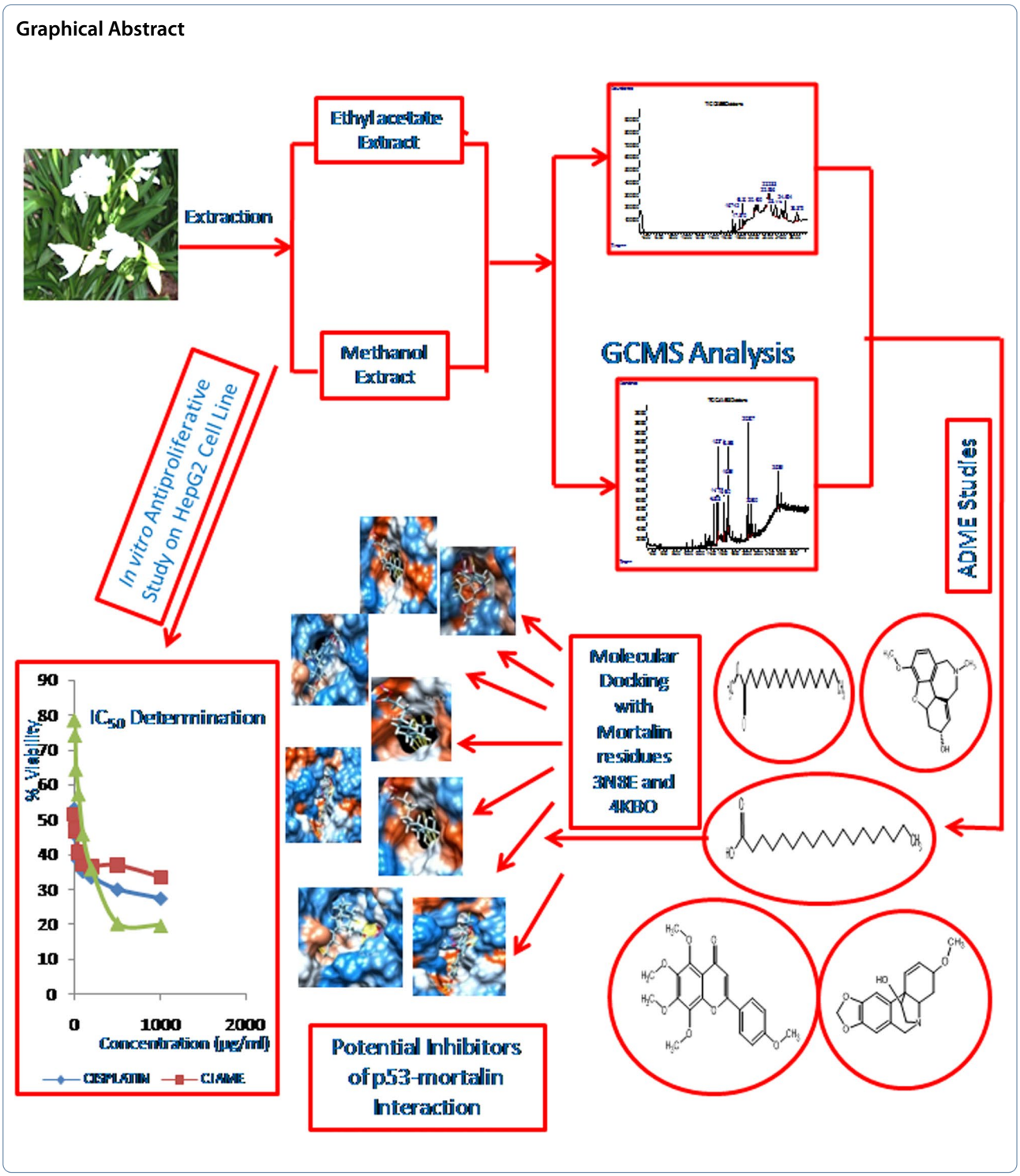

Background

Plants are used to cure various ailments by traditional medicinal practitioners. Despite the efficacy of synthetic pharmaceuticals, many people prefer plant-based treatments over synthetic drugs since they are more natural and have fewer side effects [1]. Herbal medicines contain a complex combination of phytochemicals with a wide range of structures. These compounds

are responsible for the pharmacological properties displayed by plants [2]. Plants have produced many compounds of pharmacological importance. Examples include the anticancer drugs vinblastine and vincristine (from *Catharanthus roseus* (L.) G. Don), taxol (from *Taxus brevifolia Nutt),* etoposide (a semi-synthetic analogue of podophyllotoxin, isolated from *Podophyllum* species), and topotecan (an analogue of camptothin, isolated from *Camptotheca acuminata* Decne) [3, 4]. Plants will continue to be sources of lead compounds for fuelling the drug development pipeline, given that only a small fraction of the world's biodiversity has been investigated for bioactivity so far.

Fast biological and chemical screening methods are used in the quest for novel bioactive chemicals from plants. Chemical screening involves the online characterization of bioactive compounds in crude extracts of plants using various hyphenated techniques such as high-performance liquid chromatography coupled with mass spectrometry (LC–MS or LC–MS-MS), high-performance liquid chromatography coupled with UV photodiode array detection (LC-DAD-UV), and gas chromatography coupled with mass spectrometry (GC–MS)[5]. The chemical screening approach provides preliminary information on chemical constituents and potential pharmacological properties of the plant under investigation. The GC–MS technique has various advantages. These include high chromatographic separation power, reliable quantification methodologies, and identification of metabolites with high accuracy [6]. GC separates low molecular weight metabolites that are either volatile or can be transformed into volatile and thermally stable molecules by chemical derivatization. To improve volatility and reduce the polarity of polar hydroxyl (-OH), amine (-NH_2), carboxyl (-COOH), and thiol (-SH) groups, derivatization is often required [7]. In the biological screening approach, extracts of plants are subjected to various assays to determine their potential pharmacological properties.

Hepatocellular carcinoma (HCC) is the world's third leading cause of cancer death. Around 953,000 cases of liver cancer were reported worldwide in 2017, with 819,000 people dying from the disease [8]. In Sub-Saharan Africa, liver cancer was the second leading cause of cancer-related deaths in males and the fourth in women in 2020. The high prevalence of risk factors (such as alcohol consumption and cigarette smoking, aflatoxin B1 pollution, and hepatitis B and C infection) in Sub-Saharan Africa contributes to the region's high HCC rate. HCC has a poor prognosis in black Africans, with a median survival rate of 3–4 months [9]. Mortalin, an hsp70 chaperone protein, heightens liver cancer incidence by sequestering the p53 tumor suppressor protein into the cytoplasm, thereby inhibiting its cellular functions. p53 functional inactivation extends the longevity of normal human cells and accelerates the malignancy of cancer cells [10]. Inhibiting the mortalin–p53 interactions has therefore been proposed as a viable anticancer strategy against HCC. Many compounds isolated from plants are known inhibitors of p53–mortalin interaction. For example, Pham et al. [11] identified nine triterpenes (ailanthusin A, ailanthusin B, ailanthusin C, ailanthusin D, ailanthusin F, ailanthusin G, schisanlactone C, ailanaltiolide I, and ursolic acid) as promising p53–mortalin inhibitors, based on their binding affinity and drug-like and pharmacokinetic properties; campersterol, a constituent of black rice (*Oryza sativa* L.), was identified as an inhibitor of the p53–mortalin interaction [12]; solasonine, a steroidal glycoalkaloid from Solanaceae, is a potent inhibitor of p53–mortalin interactions [10]; embelin, a natural quinone found in the fruits of *Embelia ribes* Burm. f., inhibits mortalin–p53 interactions and activates p53 protein in tumor cells [13], and salvianolic acid B, a caffeic acid phenethyl ester analog, binds to mortalin [14].

Several *Crinum* species are used in ethnomedicine for treating various cancer types. Also, literature search shows that several *Crinum* species are active against many cancer cell lines. For example, bulb extract of *Crinum ornatum* (L.f. ex Aiton) Bury is used in traditional medicine to treat breast cancer [15], *Crinum bulbispermum* ((Brum. f.) Milne-Redhead and Schweickerdt) is used by Zulu, Sotho, and Tswana people to treat tumors, and in Madagascar, *Crinum powellii* Baker Handb. is used to treat abscesses and tumors [16]. Hot water extracts of *C. asiaticum* Linn leaves inhibited calprotectin-induced cytotoxicity in MM46 mouse mammary carcinoma cells [17]; palmilycorine, isolated from *C. asiaticum*, had an inhibitory effect on the viability of ascites tumor cells [18]. The polysaccharide, *CAL-n*, isolated and purified from dried fresh seeds of *C. asiaticum* had an IC_{50} value of 128.07 μg/mL when screened for anti-tumor activity against the HepG2 cell line [19]. Oral intake of hot aqueous extract of *C. delagoense I. Verd.* bulbs was reported as a cure for human cancer; crinamine, lycorine, and 6-hydroxycrinamine isolated from the *C. delagoense* bulbs showed activity against BL-6 mouse melanoma cells [20]. Shawky and co-workers [16] showed that Genapol X-80 and DES-3 extracts of *Crinum powellii* and *C. bulbispermum* bulbs had enhanced activities against HepG2 and HCT 116 cell lines. Perlolyrine, isolated from the leaves of *Crinum latifolium L.,* showed significant cytotoxicity against five human cancer cell lines, including KB, HepG2, MCF7, SK-Mel2, and LNCaP with the IC_{50} values ranging from 22.12 ± 2.80 to 28.45 ± 3.75 μM [21].

Traditional healers in southwestern Nigeria use the bulb and leaves extracts of *Crinum jagus* (J. Thomp.) Dandy, to treat cancer. Ka et al. [22] reported the isolation of gigantelline, gigantellinine, gigancrinine, lycorine-, cherylline-, galanthamine-, and crinine-type alkaloids from *C. jagus* bulb. Another study identified the alkaloids: lycorine, hippadine, ambelline, 3-O-demethyltazettine, acetylambelline, crinanine acetate, crinine, acetylcaranine, caranine, O-methylmacronine, crinine acetate, epinorgalanthamine, trispheridine, 3-epimacronine, and voacangine in the bulb of *C. jagus* [23]. Previous studies indicate that *C. jagus* bulb extracts have hypoglycemic [15], antiplasmodial, and antimycobacterial [24, 25] properties. Methanol extract of *C. jagus* bulbs demonstrated cytotoxic and antiproliferative properties in brine shrimp, *Allium cepa*, and Sorghum bicolor radical growth inhibition assays [26]. While extensive studies have been carried out on the bulb of the *C. jagus*, no information exists in the literature on the phytoconstituents of the leaves of the plant. Also, there are no published studies on the anticancer potential of *C. jagus* leaf extracts. This research screens ethyl acetate and methanol extracts of the leaves of ***C. jagus*** for antiproliferative properties against HepG2 cell line. In addition, the phytoconstituents in the extracts would be characterized and analyzed for drug-like properties. Finally, molecular docking studies will be conducted on the phytoconstituents to identify potential p53–mortalin binding inhibitors.

Methods

Collection and extraction of plant samples

Fresh *C. jagus* leaf samples were collected at the Botanical Gardens of the University of Ibadan and identified by Mr. Kayode Owolabi, a taxonomist of the Garden. The plant sample was deposited at the University's herbarium and was assigned the voucher number: UIH-22441. The leaves were dried under mild sunlight for three weeks and ground into powder. A 1 kg portion of the powdered material was de-fatted by maceration in 2.5 L of n-hexane for 72 h. The mixture was filtered, and the filtrate was discarded. The residue was used for the subsequent steps of the extraction process. The ethyl acetate extract was obtained by soaking the residue in 2.5 L of ethyl acetate for 72 h. The filtrate (extract) was separated from the residue by filtration and concentrated using a rotary evaporator. The ethyl acetate extract was assigned the code CJLEE. The methanol extract was obtained from the residue (obtained after extraction with ethyl acetate) by maceration in methanol for 72 h. The mixture was filtered, and the filtrate (extract) was concentrated using a rotary evaporator. The methanol extract was assigned the code CJLME. The solvents used for extraction (hexane, ethyl acetate, and methanol) were procured from Sigma-Aldrich.

Anticancer screening of selected extracts in HepG2 cell line

The HepG2 cell line was purchased from American Type Culture Collection (ATCC) (USA). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (supplemented with 10% fetal bovine serum (FBS) and 1% of penicillin–streptomycin) and were incubated at 37 °C under 5% CO_2 in a humidified atmosphere. Cell culture, at a concentration of 2×10^3 cells/mL, was prepared and plated (100 µL/well) onto 96-well plates. Different concentrations (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 20 µg/mL, and 1 µg/mL) of the extracts (diluted in FBS-free medium) and the Standard (cisplatin) were introduced, as appropriate, into each well. After 72 h, MTT solution was added followed by incubation for 3 h. Dimethyl sulfoxide (DMSO) (100 µl) was used to dissolve the purple formazan crystals formed. Thereafter, the optical density of the plant extract was measured at a wavelength of 570 nm on a microplate reader (Tecan, Switzerland). The percentage cell viability was determined using the expression:

$$\text{Cell viability} = \frac{\text{Absorbance of sample (mean)}}{\text{Absorbance of Control (mean)}} \times 100\%$$

The percentages of viable cells were plotted against different extract concentrations. Cytotoxicity was recorded as the IC_{50} (the drug concentration causing 50% growth inhibition of the tumor cells) [27]. The MTT reagent and DMSO were purchased from Sigma-Aldrich.

Gas chromatography–mass spectrometry analysis of extracts

Derivatization of extract into trimethyl silyl (TMS) forms

The extracts were derivatized into Trimethyl Silyl (TMS) form as described by [28]. A 3 mg portion of each extract was treated with dry pyridine (60 µL) and bis(trimethylsilyl)trifluoroacetamide in 1% trimethylchlorosilane (100 µL). The mixture was heated at 70 °C for 30 min. GC–MS is used to analyze an aliquot of the resultant solution. Pyridine and bis(trimethylsilyl) trifluoroacetamide in 1% trimethylchlorosilane were obtained from Sigma-Aldrich.

Derivatization of extract into fatty acid methyl ester (FAME) forms

The extracts were derivatized into methyl ester forms using procedures described by [29]. A 5 mg portion of each extract was weighed into test tubes. Each extract was heated with a $CH_3OH/H_2SO_4/CHCl_3$ (1.7:0.3:2.0 v/v/v, 4 mL) mixture for 90 min at 90 °C. After cooling,

1 mL of water was added and vigorously shaken. Using a Pasteur pipette, the $CHCl_3$ layer (lower part) was gently removed and transferred to GC vials. GC–MS was used to analyze the prepared FAME derivatives. Methanol, chloroform, and tetraoxosulfate (VI) acid were obtained from Sigma-Aldrich.

GC–MS analysis of derivatized extracts

An Agilent 7890 Gas Chromatograph coupled to an Agilent 5975C mass selective detector in electron impact mode (ionization voltage, 70 eV) was used to analyze the derivatized samples. An Agilent Chrompack CP-Wax 52 CB capillary column was used to separate the sample constituents. The column had an internal diameter of 0.32 mm, a column length of 30 m, and a film thickness of 0.25 m, respectively. A 1.0 µL portion of the diluted sample was injected splitless into the chromatograph at a temperature of 250 °C. Helium, flowing at 5 mL/min, was the carrier gas. The inlet pressure was 12.936 p.s.i. The column oven temperature was gradually increased from 50 to 240 °C at 8 °C/min. The total time spent on hold was 5 min. The sample contents were identified by comparing their spectra to the Mass Spectral Library of the National Institute of Standards and Technology (NIST) on the GC–MS database (NIST 14L).

Prediction of drug-likeness of phytoconstituents

The ADME-related physicochemical properties and the drug-likeness of the bioactive compounds detected in *C. jagus* extracts were predicted by pasting the SMILES format of each compound in the SwissADME online web server (https://www.swissadme.ch). For drug likeliness, the molecular parameters such as molecular weight, hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), lipophilicity log (log P), and topological polar surface area (TPSA) were assessed. The cutoff values of the properties were set by Lipinski's rule of five (ROF). The physicochemical properties analyzed include gastrointestinal (GI) absorption and permeability of the blood–brain penetration barrier [30].

Molecular docking analysis

Ligand preparation

After evaluating the bioactive molecules identified in the extracts for drug-likeness, crinamine, galanthamine, tangeretin, hexadecanoic acid, and hexadecanoicacid methyl ester were selected for docking with the target proteins. Withaferin A and withanone, which are known inhibitors of mortalin, were chosen as standards against which the binding properties of the phytoconstituents were measured [31]. Structures of crinamine, galanthamine, tangeretin, hexadecanoic acid, hexadecanoic acid methyl ester, withaferin A, and withanone were obtained from the PubChem database and saved in the SDF format. Before docking, these structures were converted into mol2 format using the Open Babel software [32].

Protein preparation

There are two possible binding sites of mortalin into p53: the peptide binding domain, matching with PDB ID: 3N8E (residues 439–597), and the residues 253–282 domain that matched with PDB ID: 4KBO [33, 34]. These were the two models selected for the current study which were obtained from the Protein Data Bank.

Blind docking

To investigate the interaction between crinamine, galanthamine, tangeretin, hexadecanoic acid, and hexadecanoicacid methyl ester and the target proteins, docking was carried out using the SwissDock Server (http://www.swissdock.ch/) [35]. The proteins and ligands, (in the PDB and mol2 formats, respectively) were uploaded as appropriate. A link containing each docking result was sent to the user's email. All the possible binding modes for each ligand were generated by SwissDock. The most favorable binding modes at a given pocket were clustered and saved in an output file referred to as the prediction file which contains information such as estimated binding free energy, cluster rank, element, and the full fitness score. The lowest energy model was considered to be the most favorable interaction. After docking, Chimera was used to visualize the interactions between the receptors and the ligands. The amino acids interacting with the ligands, the specific atoms involved, and the hydrogen bonds formed were identified [36].

Results

To investigate the extracts for antiproliferative properties, the percentage of viable cells was plotted against different extract concentrations, as shown in Fig. 1. From the graphs, IC_{50} values of 5 µg/mL, 5 µg/mL, and 70 µg/mL were obtained for the standard (cisplatin), CJLME, and CJLEE, respectively. The error bars represent the standard deviation of the data sets.

The compounds identified in the ethyl acetate and methanol extracts of *C. jagus* leaves are neophytadiene (1), hexadecanoic acid methyl ester (2), n-hexadecanoic acid (3), 3′,4′,5,6,7,8-hexamethoxyflavone (nobiletin) (4), eicosane (5), 4′,5,6,7,8-pentamethoxyflavone (tangeretin) (6), stearic acid (7), eicosanoic acid (8), docosanoic acid (9), hexadecanoic acid ethyl ester (10), galanthamine (11), and crinamine (12). The peak areas and the elution times of each component are shown in Tables 1, 2, 3, 4. The GC–MS chromatograms of the

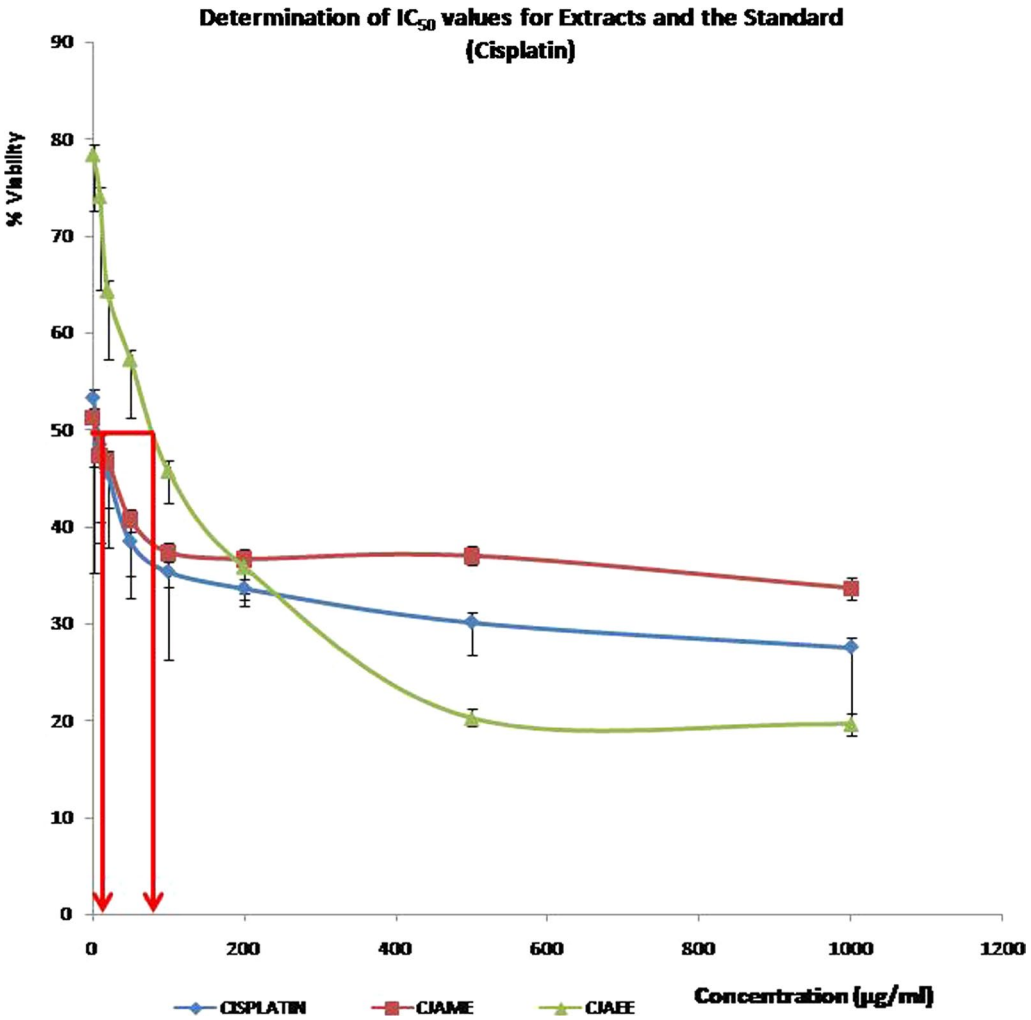

Fig. 1 Plots of percentage viability of HepG2 cells against different extract concentrations

Table 1 Compounds detected in TMS-derivatized CJLEE

t_R(min.)	Name of compound	Peak area (%)
16.739	Neophytadiene	4.22
17.872	Hexadecanoic acid methyl ester	2.68
18.336	n-hexadecanoic acid	9.22
22.233	3′,4′,5,6,7,8-hexamethoxyflavone (Nobiletin)	16.78
23.177	Eicosane	23.08
26.375	4′,5,6,7,8-pentamethoxyflavone (Tangeretin)	15.02

Table 2 Fatty acids in FAME-derivatized CJLEE (detected as methyl esters)

t_R(min)	Name of compound	Peak area (%)
15.933	Hexadecanoic acid	27.00
17.907	Stearic acid	18.62
19.709	Eicosanoic acid	2.54
21.386	Docosanoic acid	3.86

Table 3 Compounds detected in TMS-derivatized CJLME

t_R(min.)	Name of compound	Peak area (%)
14.502	Hexadecanoic acid methyl ester	6.29
14.954	n-hexadecanoic acid	17.91
15.200	Hexadecanoic acid ethyl ester	17.37
20.310	Galanthamine	19.43
20.859	Crinamine	7.94

Table 4 Fatty acids in FAME-derivatized CJLME (detected as methyl esters)

t_R(min.)	Name of compound	Peak area (%)
15.973	Hexadecanoic acid	25.02
16.654	Hexadecanoic acid ethyl ester	9.73
17.947	Stearic acid	18.49
21.426	Docosanoic acid	3.90

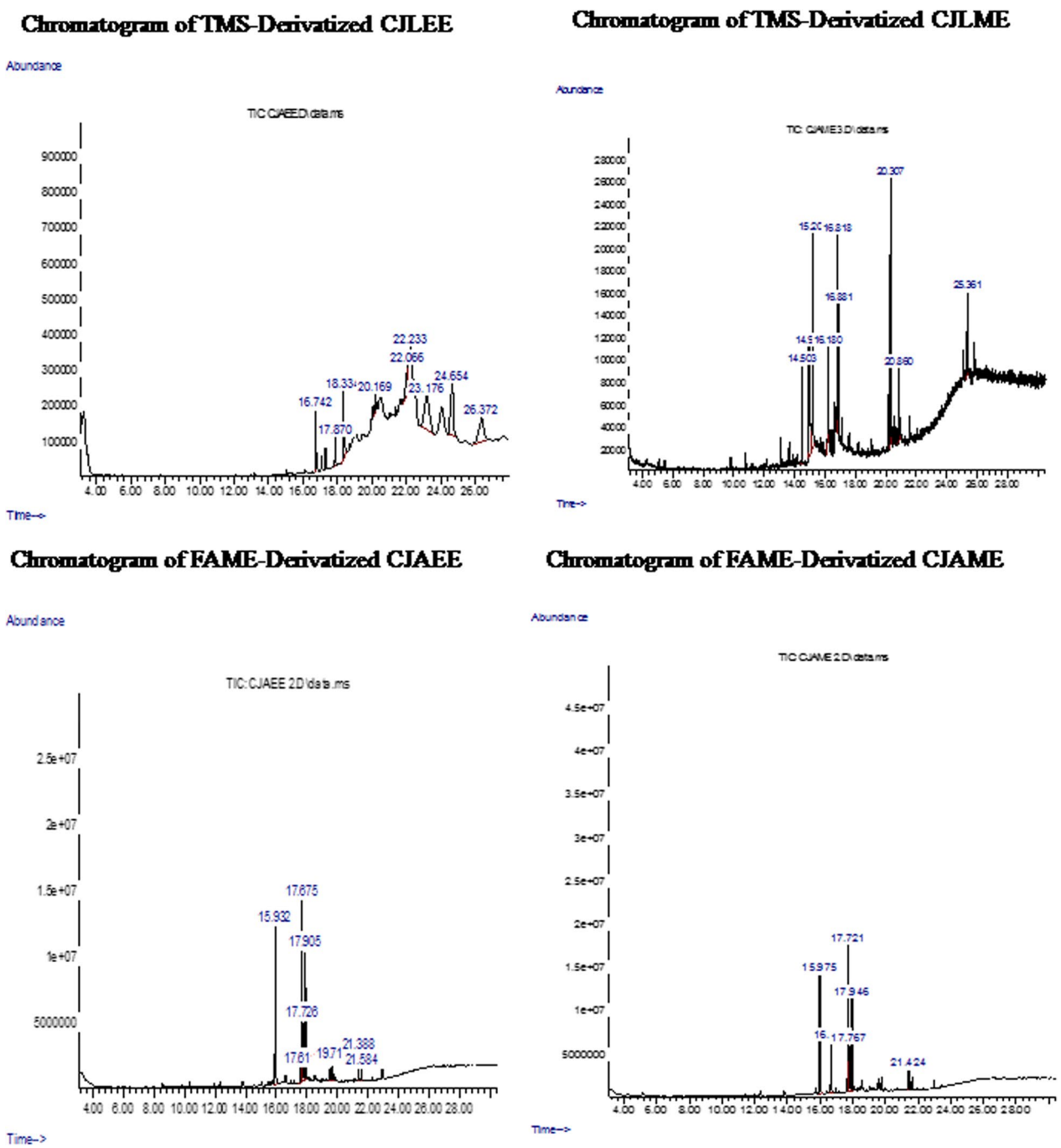

Fig. 2 GC–MS chromatograms of the derivatized ethyl acetate and methanol extracts

derivatized ethyl acetate and methanol extracts are shown in Fig. 2. The structures of the compounds are shown in Fig. 3.

Tables 5 and 6 show the physicochemical and pharmacokinetic parameters of the compounds detected in the ethyl acetate and methanol extracts.

Based on the physicochemical and pharmacokinetic properties of the compounds, hexadecanoic acid, methyl ester, n-hexadecanoic acid, tangeretin, galanthamine,

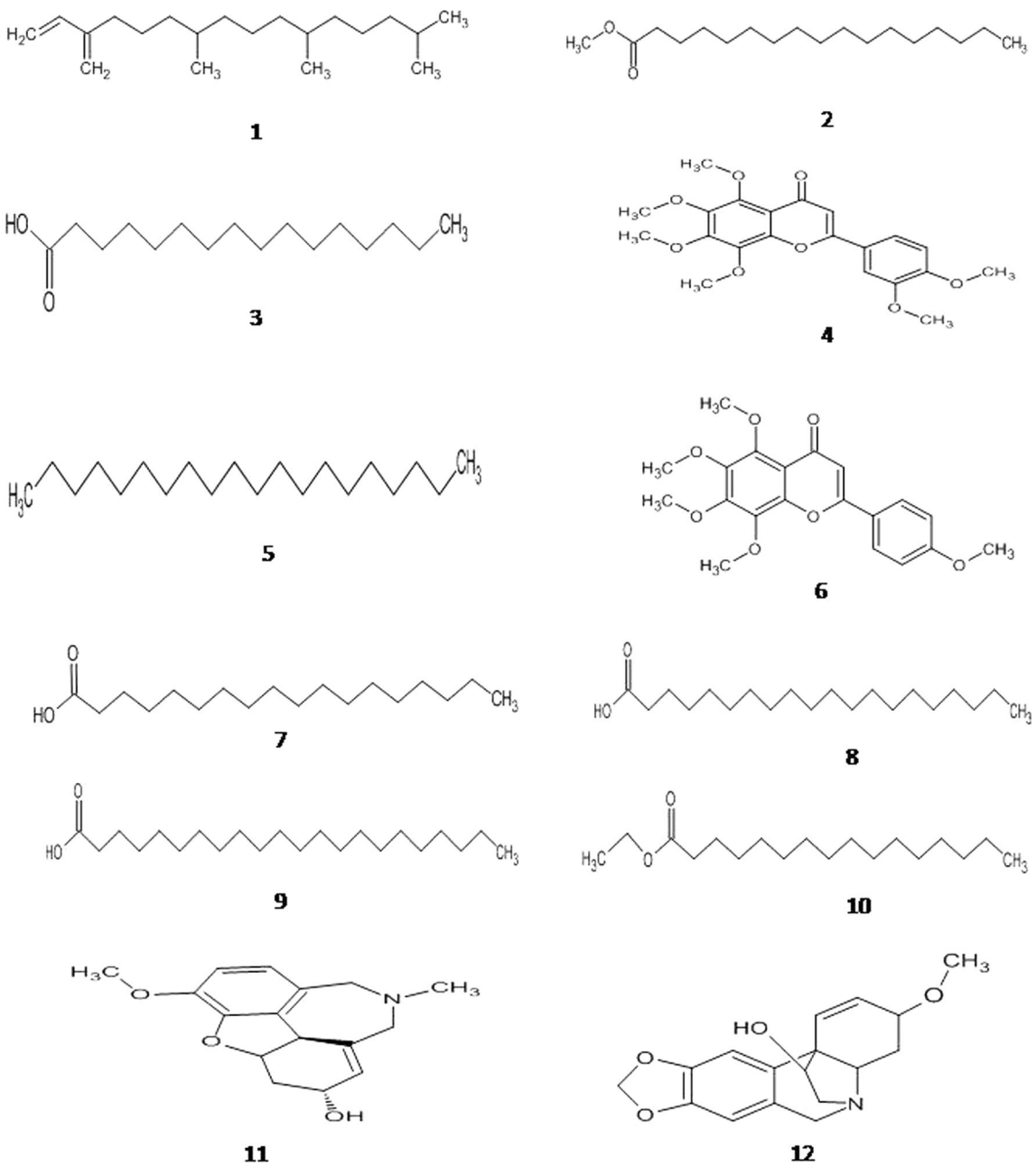

Fig. 3 Structures of phytoconstituents detected in ethyl acetate and methanol extracts of *Crinum jagus* Leaves

and crinamine were selected for docking with the mortalin residues, 3N8E and 4KBO, using the SwissDock Web server (http://www.swissdock.ch). The aim was to determine if any of the compounds could play a role in disrupting the mortalin–p53 interaction.

Crinamine, galanthamine, tangeretin, hexadecanoic acid, and hexadecanoic acid methyl ester bound with the mortalin residues 439–597 (PBID:3N8E) with energy of − 7.35, − 7.06, − 7.91, − 8.21, and − 8.21 kcal mol^{-1}, respectively (Table 7). The binding energy of tangeretin, hexadecanoic acid, and hexadecanoic acid methyl ester was very close to that of withaferin A (which had a binding energy of − 8.29 kcal mol^{-1}). Hexadecanoic acid and hexadecanoic acid methyl ester bind better to the protein residue (3N8E) than withanone (with a binding energy of − 8.14 kcal mol^{-1}). Also, from the full fitness scores, tangeretin, hexadecanoic acid, and hexadecanoic acid methyl ester had more favorable binding modes than withaferin

Table 5 Physicochemical parameters computed for bioactive compounds identified in *Crinum jagus* extracts

Compound	MW	iLog P	TPSA (A^2)	HBD	HBA	Lipinski RO5 violation
Neophytadiene	278.52	5.05	0.00	0	0	0
Hexadecanoic acid methyl ester	270.45	4.41	26.30	0	2	0
n-hexadecanoic acid	256.42	3.85	37.30	1	2	0
3′,4′,5,6,7,8-hexamethoxyflavone (Nobiletin)	580.53	3.00	184.97	4	14	2
Eicosane	282.55	5.64	0.00	0	0	0
4′,5,6,7,8-pentamethoxyflavone (Tangeretin)	372.37	3.71	76.36	0	7	0
Stearic acid	284.48	4.30	37.30	1	2	0
Eicosanoic acid	312.53	4.56	37.30	1	2	0
Docosanoic acid	340.58	5.26	37.30	1	2	0
Hexadecanoic acid ethyl ester	284.48	4.65	26.30	0	2	0
Galanthamine	287.35	2.66	41.93	1	4	0
Crinamine	301.34	2.95	51.16	1	5	0

MW molecular weight (< 500), iLog Po/w = octanol/water partition coefficient (2.0–6.5), *PSA* polar surface area, *HBD* hydrogen bond donor (< 5), and *HBA* hydrogen bond acceptor (< 10)

Table 6 Pharmacokinetic properties of phytoconstituents identified in *Crinum jagus* extracts

Compound	GI	BBB	Pgp	BS	WLOGP	SA
Neophytadiene	Low	No	Yes	0.55	7.17	4.08
Hexadecanoic acid methyl ester	High	Yes	No	0.55	5.64	2.53
n-hexadecanoic acid	High	Yes	No	0.85	5.55	2.31
3′,4′,5,6,7,8-hexamethoxyflavone (Nobiletin)	Low	No	Yes	0.17	0.69	6.10
Eicosane	Low	No	No	0.55	8.05	2.72
4′,5,6,7,8-pentamethoxyflavone (Tangeretin)	High	Yes	No	0.55	3.50	3.74
Stearic acid	High	No	No	0.85	6.33	2.54
Eicosanoic acid	Low	No	No	0.85	7.11	2.77
Docosanoic acid	Low	No	No	0.85	7.89	3.00
Hexadecanoic acid ethyl ester	High	No	No	0.55	6.03	2.80
Galanthamine	High	Yes	Yes	0.55	1.32	4.57
Crinamine	High	Yes	No	0.55	0.65	4.82

GI gastrointestinal absorption, *BBB* blood–brain barrier, *Pgp* permeability glycoprotein, *BS* bioavailability score, *SA* synthetic accessibility

A and withanone. The docking results showed hydrogen bonds and other weaker interactions exist between the ligand and 3N8E. The docking poses of crinamine (A), galanthamine (B), hexadecanoic acid (C); hexadecanoic acid methyl ester (D), tangeretin (E), withaferin A (F), and withanone (G) against 3N8E are shown in Fig. 4. The hydrogen bonds are shown in purple while yellow bonds represent other clashes/interactions between the ligands and protein. Table 7 shows the docking results of the ligands with the protein residue.

Crinamine, galanthamine, tangeretin, hexadecanoic acid, and hexadecanoic acid methyl ester had binding energies of − 6.99, − 7.47, − 7.25, − 7.66, − 7.45 kcal mol^{-1}, respectively, with 4KBO. Withaferin A and withanone had binding energy of − 7.68 kcal mol^{-1} and − 7.59 kcal mol^{-1}, respectively (Table 8). The binding energy of galanthamine, hexadecanoic acid, and hexadecanoic acid methyl ester was very close to those of withaferin A and withanone. Hexadecanoic acid binds better with the protein than withanone. In addition, galanthamine, hexadecanoic acid, and hexadecanoic acid methyl ester (with full fitness scores of − 1947.2039, − 2018.7064, and − 2003.8347 kcal mol^{-1}, respectively) had better binding modes than withaferin A and

Table 7 Docking results of mortalin residue 3N8E with selected phytoconstituents

Compound	Binding affinity (kcal/mol)	Full fitness	H-bonding interactions (Ligand…Protein)	Length (A°)
Crinamine	− 7.35	− 1896.508	GLU448 H12…O	4.296
			VAL453 O…HN	6.370
			GLY452 O…HN	4.624
			LYS485 O2…HN	5.182
Galanthamine	− 7.06	− 1926.987	VAL453 N…O	6.590
			GLY587 O…HN	6.6828
			GLY587 O2…HN	6.784
			GLU580 H17…O	6.840
			GLU580 H13…HA	2.295
			GLU580 H18…O	2.572
Tangeretin	− 7.91	− 1866.2689	GLY587 O3…HN	6.071
			GLU586 O3…HN	6.784
			LEU450 O…HN	2.943
			VAL453 O2…HN	6.242
Hexadecanoic acid	− 8.21	− 2000.716	ALA474 O…HN	7.228
			GLY451 O1…HN	2.337
Hexadecanoic acid methyl ester	− 8.21	− 2000.716	ALA474 O…HN	7.228
			GLY451 O1…HN	2.337
Withaferin A	− 8.29	− 1692.9403	GLY514 O3…HN	6.752
			GLU483 O4…HN	5.859
			GLU483 O…H37	3.153
			LYS576 O…H29	6.688
			GLU577 O…H29	6.073
			ARG578 O…H29	7.258
			GLU580 O…H29	2.608
Withanone	− 8.14	− 1668.8639	GLY514 O4…HN	6.817
			SER473 O2…HN	4.101
			GLY587 O3…HN	7.181
			GLU483 O5…HN	5.952

withanone (with full fitness scores of − 1702.6406 and − 1665.646 kcal mol^{-1}, respectively). The ligands had hydrogen bonds (and other weaker) interactions with the protein residue. Different docking poses of crinamine (A), galanthamine (B), hexadecanoic acid (C), hexadecanoic acid methyl ester (D), tangeretin (E), withaferin A (F), and withanone (G) against 4KBO are shown in Fig. 5. The hydrogen bonds are shown in purple while yellow bonds represent other clashes/interactions between the ligands and protein.

Discussion

After de-fatting, the dried leaf samples were extracted sequentially using solvents of varying polarities (ethyl acetate is less polar than methanol), thereby separating the phytoconstituents in the extracts based on their polarities. The extracts under investigation will, therefore, exhibit varying antiproliferative properties. The graph obtained by plotting percentage of viable cells against different extract concentrations showed that the cell viability decreased with the increasing concentration of the extracts (Fig. 1). The results obtained from previous studies have shown similar trends [37]. The IC_{50} values obtained indicate that the antiproliferative property of CJLME is comparable to that of the standard drug (cisplatin). The National Cancer Institute Guidelines (USA) stated that extracts with $IC_{50} < 30$ µg/mL are antiproliferative agents [38]. CJLME possesses significant antiproliferative properties.

Literature reports show that many of the compounds identified in the ethyl acetate and methanol extracts possess anticancer properties. Nobiletin and tangeretin have antiproliferative activities against gastric cancer, leukemia (HL-60), squamous cell carcinoma (HBT43), B16 melanoma, human lung carcinoma (A549), and T-cell leukemia (CCRF-HSB-2) cell lines [39]. In addition, n-hexadecanoic acid had an IC_{50} of 0.8 µg/mL against human colorectal carcinoma (HCT-116) cells [40]. Similarly, Crinamine exhibited significant cytotoxic against human breast cancer (BCA-1), human fibrosarcoma (HT-1080), human lung cancer (LUC-1), human melanoma (MEL-2), and human colon cancer (COL-1) and cervical cancer (SiHa) cell lines [41, 42]. These compounds could

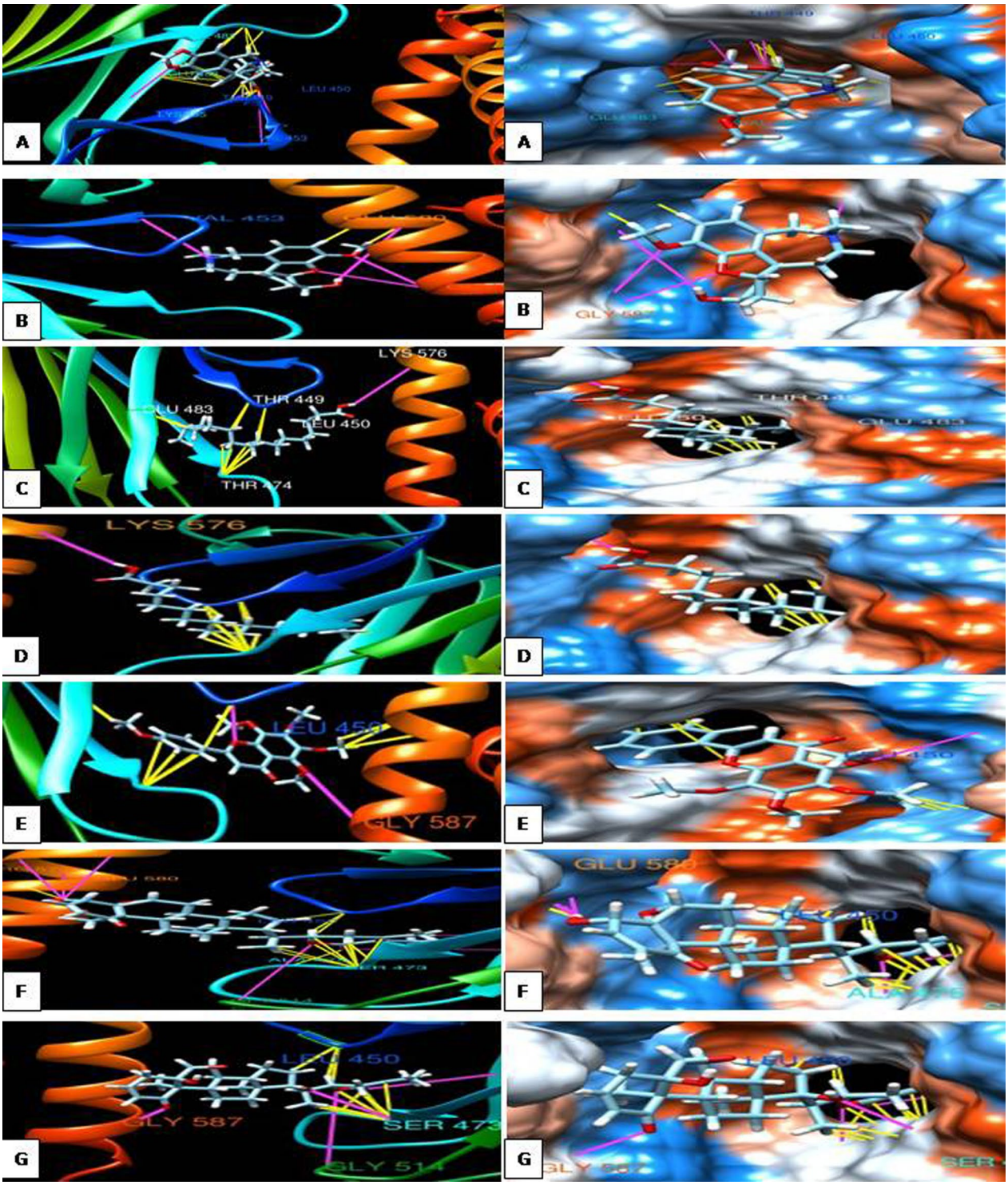

Fig. 4 Docking poses of phytoconstituents against mortalin residue, 3N8E

be partly responsible for the anticancer properties demonstrated by the leaves of *C. jagus*.

A potential drug candidate must possess a good absorption, distribution, metabolism, and excretion (ADME) profile. With advances in science, it is now possible to predict the ADME properties of new drug candidates in silico [43]. Lipinski and co-workers [44] proposed that a compound must satisfy certain conditions (referred to as

Table 8 Docking results of mortalin residue 4KBO with selected phytoconstituents

Compound	Binding affinity (kcal/mol)	Full fitness	H-bonds	Length (A°)
Crinamine	− 6.99	− 1910.8486	ARG309 H12…O GLU310 H12…O ALA311 H12…O TIP3 605 O2…H1	6.455 6.122 7.034 5.867
Galanthamine	− 7.47	− 1947.2039	ARG309 H17…O ALA312 H17…O PHE278 O1…HN	7.275 2.978 1.422
Tangeretin	− 7.25	− 1874.5452	THR63 O2…HG1	6.741
Hexadecanoic acid	− 7.66	− 2018.7064	ILE211 O…HN GLN203 O…H31 ALA204 O…H31	6.994 6.825 5.785
Hexadecanoic acid methylester	− 7.45	− 2003.8347	THR390 O1…NH GLY388 O1…NH TIP3 604 O1…H2 TIP3 605 O1…H2 THR249 O1…NH	5.894 2.293 5.035 2.299 7.014
Withaferin A	− 7.68	− 1702.6406	MET103 H29…O ASP279 O4…HN ARG391 O5…HN GLY388 O5…HN	6.819 6.202 6.852 2.894
Withanone	− 7.59	− 1665.646	GLU276 H23…O	4.414

the rule of 5 (Ro5)) before it can be a viable drug candidate: the hydrogen bond donors in the compound ≤ 5, the hydrogen bond acceptors ≤ 10, the octanol/water coefficient (log P) ≤ 5, and the molecular weight must be less than 500. When taken together with Lipinski's Ro5, polar surface area (PSA) can indicate the candidate's bioavailability [45]. Orally bioavailable compounds with a PSA less than $140A^2$ exhibit better intestinal absorption [46], while compounds with PSA values less than $70A^2$ can penetrate the blood–brain barrier [45]. From Table 5, Compound 4 (nobiletin) violated two of Lipinski's Ro5 requirements (molecular weight and HBA) and therefore has poor potential as a drug candidate. The compound (nobiletin) would also likely possess a low bioavailability since its PSA value is above 140 A^2, and was therefore excluded from further study.

Table 6 shows the results of the pharmacokinetics investigations of the selected phytoconstituents detected in the extracts. Drugs must be able to transit across biological membranes seamlessly [46]. A sufficient intestinal absorption of a drug has a bearing on its action and affects its absorption, distribution, and elimination in the body [46]. In addition, a drug must be able to cross the blood–brain barrier (BBB) to exert therapeutic actions on the brain. BBB acts as a sieve that prevents the access of polar molecules to the brain. From the results obtained (Table 6), only compounds hexadecanoic acid methyl ester (2), n-hexadecanoic acid (3), tangeretin (6), galanthamine (11), and crinamine (12) have high gastrointestinal absorption and could transit across the blood–brain barrier. Galanthamine (11) is a substrate for Permeability glycoprotein (Pg-p), an efflux transporter pump found in the cell membrane which is responsible for conveying drugs away from the cell membrane and cytoplasm which causes therapeutic failure when the concentration of the drug is reduced [47]. An optimal clinical drug/derivative should have high gastrointestinal permeability and low P-gp efflux liability. However, galanthamine has been retained for further study because it is a known drug used to treat certain stages of Alzheimer's disease (AD) [48]. A bioactivity score of 0.55 shows that the compounds have excellent pharmacokinetic properties [49]. Hexadecanoic acid methyl ester (2), n-hexadecanoic acid (3), tangeretin (6), galanthamine (11), and crinamine (12) are potential drug candidates for the treatment of hepatocellular carcinoma. From Tables 7 and 8, tangeretin, hexadecanoic acid, and hexadecanoic acid methyl ester showed very great potentials as inhibitors of the mortalin residue, 3N8E (Table 7), while galanthamine,

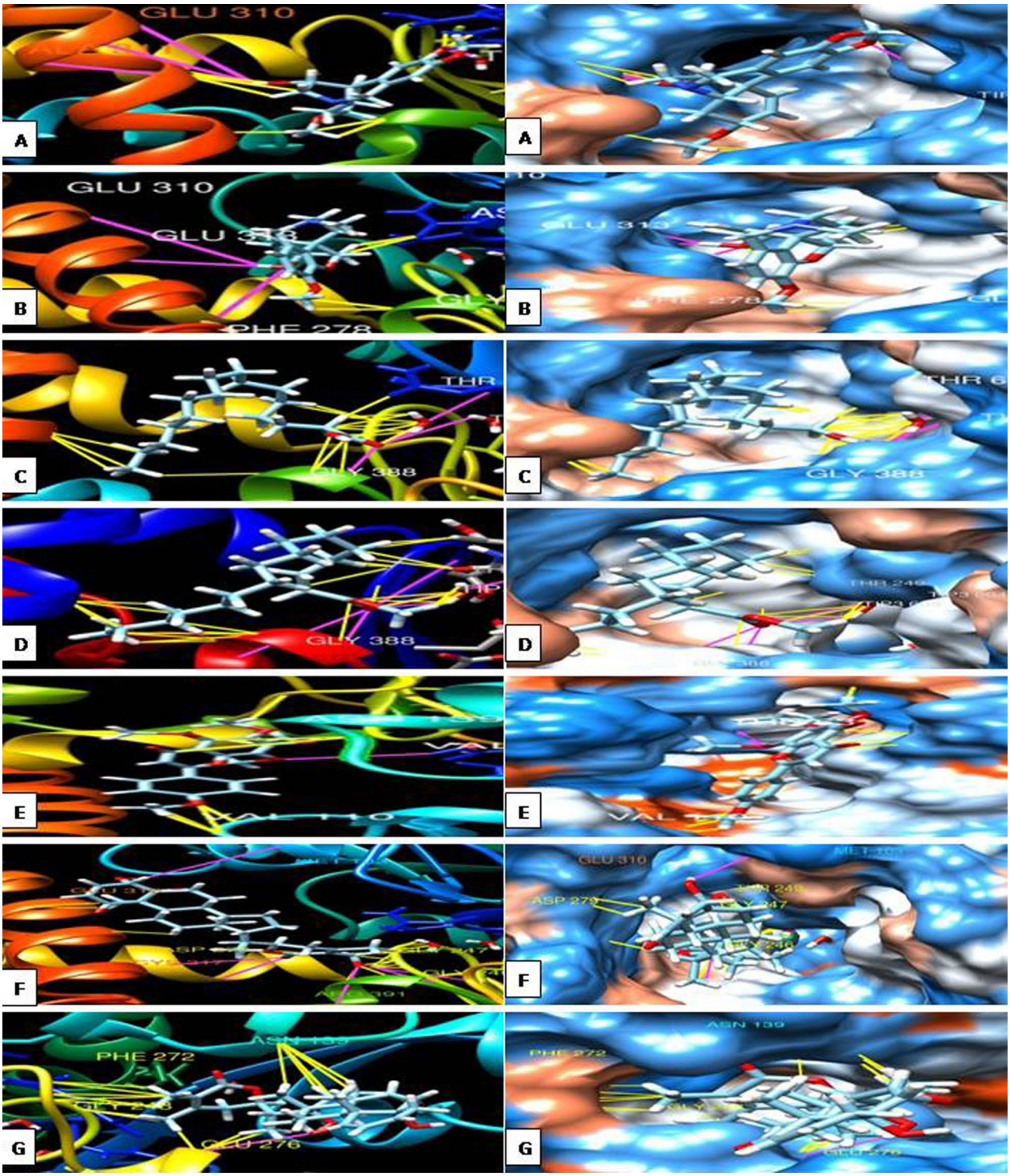

Fig. 5 Docking poses of phytoconstituents against mortalin residue, 4KBO

hexadecanoic acid, and hexadecanoic acid methyl ester showed the best potentials as inhibitors of 4KBO.

Conclusion

The results obtained from the present study showed that extracts from *Crinum jagus* possess antiproliferative properties against HepG2 cell lines. The methanol extract of the leaves demonstrated an anticancer activity comparable to that of cisplatin. Of the compounds detected in the sample by GC–MS analysis, hexadecanoic acid methyl ester, n-hexadecanoic acid, tangeretin, galanthamine, and crinamine possess druglike properties. Literature reports indicate that crimanine, tangeretin, and hexadecanoic acid detected in the extracts have anticancer properties against several human cancer cell lines; these compounds could be partly responsible for the cytotoxic properties of the extracts against the HepG2 cell line. Furthermore, n-hexadecanoic acid, hexadecanoic acid methyl ester, galanthamine, and tangeretin were identified as potential inhibitors of the p53–mortalin interaction.

Abbreviations

HCC	Hepatocellular carcinoma
HepG2	Human hepatocellular carcinoma cell line
MTT	3-(4, 5-Dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide
DMSO	Dimethyl sulfoxide
TMS	Trimethyl silyl
GCMS	Gas chromatography-mass spectrometry
FAME	Fatty acid methyl ester
NIST	National Institute of Standards and Technology
CJLEE	*Crinum jagus* leaves ethyl acetate extract
CJLME	*Crinum jagus* leaves methanol extract
HL-60	Leukemia cell line
HBT43	Squamous carcinoma cell line
B16	Melanoma cell line
A549	Human lung carcinoma cell line
CCRF-HSB-2	T-cell leukemia cell line
BCA-1	Human breast cancer cell line
HT-1080	Human fibrosarcoma cancer cell line
LUC-1	Human lung cancer cell line
MEL-2	Human melanoma cell line
COL-1	Human colon cancer cell line
SiHa	Cervical cancer cell line
ADME	Absorption, distribution, metabolism, and excretion
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
log P	Lipophilicity log
TPSA	Topological polar surface area
BBB	Blood–brain barrier
Ro5	Rule of five
GI	Gastrointestinal
3N8E	Mortalin residue (439–597)
4KBO	Mortalin residue (253–282)
Pg-p	Permeability glycoprotein
AD	Alzheimer's disease

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TT, L, MO, and BO designed the study. TT carried out the experimental studies. All authors participated in writing the manuscript. All authors read and approved the final manuscript.

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Declarations

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Completing of interests
The authors declare no conflicts of interest.

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