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Research Article

PHYTOCHEMICAL CHARACTERIZATION OF *UROCHLOA DISTACHYA* (L.) THROUGH GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS WITH *IN SILICO* AND *IN VIVO* ANTI-INFLAMMATORY ASSESSMENT IN CARRAGEENAN-INDUCED PAW EDEMA

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ABSTRACT

Objectives: The objective of this study was to evaluate the phytochemical composition and anti-inflammatory activity of chloroform and ethyl acetate extracts of *Urochloa distachya*, alongside *in silico* molecular docking analysis.

Methods: The phytochemical composition of the extracts was analyzed using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) techniques to identify bioactive compounds. The *in vivo* anti-inflammatory activity was assessed using the carrageenan-induced paw edema model in rats, where the reduction in paw edema was measured to evaluate efficacy. In addition, *in silico* molecular docking studies were conducted to determine the binding affinity of identified compounds with key inflammatory targets, including cyclooxygenase-2, 5-lipoxygenase, phosphodiesterase 4, and human peroxiredoxin 5, to explore their potential anti-inflammatory mechanisms.

Results: The GC-MS and LC-MS analyses of chloroform extract of *U. distachya* (CUD) and ethyl acetate extracts of *U. distachya* identified phytoconstituents with notable anti-inflammatory potential. In the carrageenan-induced paw edema model, the CUD demonstrated a significant reduction in paw edema in rats. *In silico* molecular docking revealed that key compounds, including Holothurin acetate, 7,8-Epoxylanostan-11-ol, 3-acetoxy-, and 7,8,12-Tri-O-acetyl-3-desoxy-ingol-3-one, exhibited strong binding affinities with inflammatory targets, ranging from −8.6 to −15.3 kcal/mol.

Conclusion: The chloroform extract of CUD exhibits significant anti-inflammatory activity, supported by both *in vivo* and *in silico* analyses. The identified phytoconstituents demonstrate strong binding affinities with key inflammatory targets.

Keywords: Phytochemicals, Gas chromatography-mass spectrometry, Liquid chromatography-mass spectrometry, Molecular docking, Anti-inflammatory activity, Carrageenan-induced, *Urochloa distachya*, Poaceae.

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INTRODUCTION

Inflammation is an intricate biological response of tissues to harmful stimuli, including irritants, damaged cells, and bacteria. This protective mechanism is crucial for initiating tissue regeneration and maintaining homeostasis. The method entails the induction of immune cells, the secretion of signaling molecules such as cytokines, and increased blood flow to the damaged region. Acute inflammation is a vital advantageous reaction to injury or infection; conversely, persistent inflammation is linked to various diseases, including aging, cardiovascular disease, cancer, and neurological disorders [1,2]. When the body experiences inflammation, whether from an infection, a mechanical injury, or a burn, its cells begin to build defense mechanisms without external interference [3]. Inflammation is usually divided into two main types, acute inflammation and chronic inflammation [4]. Acute inflammation is an instantaneous, adaptive response characterized by limited specificity, triggered by harmful stimuli such as infection and tissue injury. This advantageous reaction defends against infectious agents, including mycobacterium tuberculosis, protozoa, fungi, and other parasites [5]. The inflammatory response is crucial for providing growth factors and cytokine signals that coordinate the cellular and tissue movements essential for healing. Various studies have revealed that the inflammatory response during standard healing is marked by spatially and temporally variable patterns of different leukocyte subsets [6]. Chronic inflammation is characterized as a sustained, long-term inflammatory response that endures for several months to years. The magnitude and consequences of chronic inflammation vary depending on the injury's etiology and the body's capacity to mend and surmount the harm [7]. During the inflammatory process, there is a significant production of inflammatory biomarkers, including reactive oxygen species, reactive nitrogen species, tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, and cyclooxygenase-2 (COX-2). TNF-α, a pro-inflammatory cytokine, serves as a principal regulator of inflammatory cytokine production, influencing inflammation, immunology, cellular homeostasis, and tumor progression [8,9]. Non-steroidal anti-inflammatory drugs (NSAIDs) are mainly used for the management of pain and inflammatory disorders [10]. Examples of NSAIDs used to treat inflammation include aspirin, naproxen, ibuprofen, diclofenac, indomethacin, meloxicam, piroxicam, meclofenamate, mefenamic acid, celecoxib, and etoricoxib. The primary mechanism of action of NSAIDs involves the inhibition of the enzyme cyclooxygenase [11,12]. NSAIDs have been used for the management of inflammation. The escalating side effects, including heart attacks and strokes associated with these therapies, require the substitution of synthetic medications with plant-based alternatives [13]. Table 1 provides an overview of the current drugs used for treating inflammation, highlighting their respective limitations.

Extensive research on plant crude extracts has been undertaken in recent decades, revealing that various plants possess anti-inflammatory activities. Numerous inflammatory disorders can be efficiently managed with phytochemical substances. Alkaloids, flavonoids, polyphenols,

Table 1: Current anti-inflammatory drugs and their limitations

Sl. No.	Name of the drug	Limitation	References
1	Diclofenac	Increased risk of myocardial infarction or stroke	[14]
2	Etodolac	Gastrointestinal complaints	[15]
3	Diflunisal	Contraindicated in patients with thrombocytopenia and chronic kidney disease	[16]
4	Fenoprofen	May causes ulcer	
5	Ibuprofen	Gastrointestinal and cardiovascular complications.	[17]
6	Indomethacin	Most serious side effects such as shortness of breath, swelling, fever, pale skin, nausea, and painful urination.	[18]
7	Ketoprofen	The side effects such as Urticaria, bronchospasm, heartburn, tachycardia, constipation, and pancreatitis.	[19]
8	Meloxicam	Increase the risk of heart attack	[20]
9	Mefenamic acid	Cases of ulcer and bleeding	[21]
10	Nabumetone	The common side effects are nausea, constipation, swelling, headache, and serious side effects such as ulcers, heart attacks, blood clots, and high blood pressure.	[22]
11	Naproxen	If it is taken regularly, it causes stomach ulcers.	[23]
12	Oxaprozin	Gastrointestinal disorders are the most common adverse effects.	[24]
13	Piroxicam	The side effects include abdominal pain, headache, dizziness, diarrhea, peripheral edema, and hypersensitivity reactions.	[25]
14	Sulindac	Not recommended for cardiovascular disorder, it may produce a risk of heart attack, stroke, and blood clots.	[26]
15	Celecoxib	The severe adverse effects include congestive heart failure, liver toxicity, renal toxicity, anaphylactic reactions, and rashes.	[27]

and terpenoids are the primary chemical classes associated with anti-inflammatory, antimicrobial, anticancer, antidiabetic, antimalarial, and neuroprotective properties [28-30]. *Acacia catechu*, *Azadirachta indica*, *Caesalpinia crista*, *Cassia angustifolia*, *Coriandrum sativum*, *Cuscuta reflexa*. *Enicostema littorale, Erythrina variegata*, *Euphorbia hirta*, *Euphorbia tirucalli*, *Fagonia cretica*, *Ficus benghalensis*, *Glycyrrhiza glabra*, *Hibiscus rosa-sinensis*, *Nicotiana tabacum*, *Nigella sativa*, and *Tephrosia purpurea* are plants used for their anti-inflammatory properties [31].

Various animal models exist for evaluating anti-inflammatory potency, including carrageenan-induced paw edema, bradykinin-induced paw edema, dextran-induced paw edema, lipopolysaccharide-induced paw edema, histamine-induced paw edema, arachidonic acid-induced ear edema, 5-HT-induced paw edema croton oil/TPA-induced ear edema, oxazolone-induced ear edema, acetic acid, or compound 48/80-induced vascular permeability, and pleurisy model for acute inflammation. Subacute inflammatory models include the granuloma pouch model. The chronic inflammatory model comprises cotton pellet-induced granuloma, formalin-induced paw edema, and complete Freund's adjuvant-induced arthritis [32].

To better understand the chemical diversity and therapeutic potential of plants, phytochemical research is essential for identifying and quantifying bioactive chemicals inside plants. For this objective, several analytical methods have been used, each developed for a particular class of phytochemicals. Because of its great precision and resolution in complex plant matrices, high-performance liquid chromatography (HPLC) finds extensive usage in the separation of non-volatile chemicals, such as flavonoids and phenolics [33]. Gas chromatography-mass spectrometry (GC-MS) offers an effective method for the separation and identification of volatile compounds, including essential oils and alkaloids, due to its sensitivity and specificity [34]. Moreover, ultra-performance liquid chromatography, a sophisticated variant of HPLC, has acquired prominence for its capacity to provide expedited analyses with superior sensitivity and resolution [35]. Fourier transform infrared spectroscopy and nuclear magnetic resonance spectroscopy are essential for identifying functional groups and elucidating the structure of phytochemicals, hence improving the characterization of plant-derived substances [18,36]. With liquid chromatography-mass spectrometry (LC-MS) and ultraviolet-visible spectroscopy, investigators can look into the wide range of chemicals found in plants and find ways to use them in traditional medicine, medicines, and nutraceuticals [37].

Molecular docking is a computer method employed to anticipate the optimal orientation of one molecule when interacting with another molecule, usually a protein, to establish a stable complex. This technique is extensively utilized in pharmaceutical drug discovery and design, as it aids in comprehending molecular interactions at the atomic scale [38,39]. The primary objective of molecular docking is to ascertain the compatibility of a ligand with the binding site of a target protein and to assess the nature and strength of interactions, including hydrogen bonds, hydrophobic interactions, and electrostatic forces, that stabilize the complex [40]. COX-2 is an inducible enzyme that plays a role in the synthesis of pro-inflammatory prostaglandins, and its specific inhibition is a crucial approach to mitigating inflammation [41]. Lipoxygenase (LOX) catalyzes the synthesis of leukotrienes, potent inflammatory mediators, rendering it a significant target in conditions such as arthritis, asthma, and cancer [42]. Phosphodiesterase 4 (PDE4), responsible for the degradation of cyclic AMP, is primarily expressed in immune cells. Its inhibition has shown a reduction in inflammatory cytokine production, positioning it as a promising target for the treatment of conditions such as psoriasis, asthma, and chronic obstructive pulmonary disease [43]. Human peroxiredoxin 5 (HP5) is a crucial antioxidant enzyme that reduces peroxides and protects cells from oxidative stress, a significant factor in the pathophysiology of inflammation-related disorders [44].

Urochloa distachya, commonly known as signal grass, is a perennial grass species belonging to the family Poaceae, it is widespread in tropical and subtropical climates. It grows in grasslands, savannas, and disturbed places, especially in poor soils and degraded soils [45]. Its flexibility has rendered it a beneficial species for preventing soil erosion and enhancing soil structure, especially in regions susceptible to land degradation. Moreover, *U. distachya* is esteemed as a forage crop, providing substantial nutritional benefits for cattle, especially in tropical areas where it functions as a principal feed source during the dry season [46]. Most plants in the Poaceae family are used as traditional treatments to treat diabetes, astringents, ulcers, anthelmintic, hypersensitivity, and inflammation, and as astringents, diuretics, and also used as antioxidant agents [47]. The previous study of GC-MS analysis of methanolic and petroleum ether extract of *U. distachya* revealed the phytoconstituents includes; 7-Methyl-Z-tetradecen-1-ol acetate; 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z); 2-Naphthalenol,2,3,4,4a,5,6,7 octahydro1,4adimethyl-7-(2-hydroxy-1-methylethyl); 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex1en-1-carboxaldehyde; Spiro[4.5]decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl; 9,12,15-Octadecatrienoic

acid,2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z, Z, Z); Hexadecanoic acid, methyl ester; phytol; Campesterol; Stigmasterol; Tri-tetracontane; γ-Sitosterol; β-Amyrin; α-Amyrin; 9,19-Cyclolanostane-3,7-diolis which exhibited anti-inflammatory activity [48,49]. Furthermore, the flavonoid and steroid compounds 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl, and 26-Nor-5-cholesten-3-á-ol-25-one, identified through GC-MS analysis of the methanolic cold extract of *U. distachya*, are also documented for their anti-inflammatory activity [50].

The present study seeks to comprehensively investigate the phytochemical content and assess the anti-inflammatory properties of chloroform and ethyl acetate extract of *U. distachya* by a combination of GC-MS, LC-MS, *in vivo* anti-inflammatory assays, and molecular docking methods. The carrageenan-induced paw edema model in rats is an established method for testing the anti-inflammatory effectiveness of extracts by measuring paw swelling. Molecular docking analyses can predict the interactions of the identified phytoconstituents with significant inflammatory targets, including COX-2, 5-LOX, PDE4, and HP5.

METHODS

Chemicals and reagents

Collection and preparation of plant

The plant was authenticated by Dr. V. Ranjan (scientist, BSI, Kolkata, India), Voucher number: CNH/Tech.II/2019/77, and the plant samples were collected from the local area of Bargarh district (Hatgaon), Bargarh, Odisha, India. The whole plants were shade-dried, pulverized into powder, and preserved in an air shield container.

Extraction of plant material

The dried, powdered plants were first defatted using petroleum ether to eliminate any waxy residue. Then, the Soxhlet apparatus was used to extract the plants using chloroform and ethyl acetate in the order of their polarity. Finally, a rotary evaporator concentrated the mixture at 40°C. To measure the yield %, the dried extract was stored at 4°C. Using standard protocols, the *U. distachya* extracts were evaluated qualitatively for phytochemicals [51].

Experimental animal care

Twenty-four Wistar albino rats (7–10 weeks old, weighing 155–180 g) were acquired from M/S Chakraborty Enterprises (registration no.: 1443/PO/Bt/s/11/Committee for the Purpose of Control and Supervision of Experiments on Animals [CPCSEA], Kolkata) for anti-inflammatory study and were accommodated in cages. Before the testing, the rats had a 7-day acclimatization period to laboratory conditions, adhering to a 12-h photoperiodic at a temperature of 22±3°C. The animals were provided with conventional pellet diets and unrestricted access to water. Throughout the trial, the cages were sanitized bi-daily and maintained in a hygienic condition to avert infection. The animals received appropriate care under the ethical standards established by the International Guidelines for the Use of Laboratory Animals. The animal tests were conducted in compliance with regulations about the protection of animals utilized in scientific research. Our experiments demonstrated successful anti-inflammatory activity, as evidenced by carrageenan-induced paw edema. This validated our utilization of rats in a carrageenan-induced paw edema model to find out the anti-inflammatory effect of *U. distachya*.

Ethical statement

All experiments were conducted in complete adherence to international norms. The design protocol received approval from the Animal Ethical Committee (1376/PO/Re/S/10/CPCSEA) under project proposal numbers IAEC/01/2024 for the acute toxicity study and IAEC/03/2024 for carrageenan-induced paw edema in rats.

GC-MS analysis

GC-MS analysis was conducted with Agilent 5977 MSD technology. The mass spectrophotometer features an HP-5 MS fused silica column of 30 m×250 μm×0.25 μm. It is linked to an MSD interface. Helium served as the carrier gas, and the column flow rate was established at 1.2 ml/min. The temperature of the column varied from 60°C to 325°C (350°C), with a pressure of 11.367 psi. The whole duration of the GC operation was 40 min. A volume of 1 μL of the sample was pushed, and the mass was determined at an energy level of 70 electron volts. The quad temperature was established at 150°C, but the source temperature was configured to 230°C for a maximum length of 10 min. The chromatogram displayed the concentration of the chemical in the chloroform extract of *U. distachya* (CUD) and ethyl acetate extracts of *U. distachya* (EAUD) as a percentage [48].

LC-MS analysis

LC-MS (Agilent Technologies, USA), Direct Infusion Mass with ESI and APCI (Positive and Negative ion mode), 1260 Infinity Nano HPLC with Chipcube, and 6550 iFunnel Q-TOFs. A source temperature of 140°C was maintained for the full-scan mode (m/z 100–1200). A solvent mixture of 0.3% formic acid in methanol and a C-8 column (Phenomenex, 5 μ, 2 mm, id) were used to analyze the compounds, with a flow rate of 0.1 mL/min. All gas parameters were kept constant during the test: 6 ml/min gas flow, 25 psi nebulizing pressure, and 350°C drying gas temperature. Centrifuged at 12,000 rpm for 10 min, the CUS and EAUD extracts (0.5 g) were diluted in methanol before analysis. To determine the mass fragmentation of the compounds, the Spectrum Database for Organic Compounds (SDBS) was used [52].

Acute toxicity study

In line with the Organization for Economic Cooperation and Development (OECD) guideline 423 (Annexure 2b), an acute oral toxicity study took place [53]. Three rats from each group, CUD and EAUD, were randomly selected at a dosage of 2000 mg/kg body weight. The samples were solubilized in a solution of 1% carboxymethylcellulose (CMC) and 3% dimethyl sulfoxide. Post-treatment, the rats were monitored for any behavioral alterations during the initial 4 h, followed by an additional 48 h of inspection. The rats were monitored daily for 14 days. This study aimed to ascertain the emergence of clinical or toxicological symptoms as per the guidelines set by the OECD. All observations, including alterations in the skin, eyes, and mucous membranes, alongside behavioral patterns such as food consumption, body weight, temperature, respiration, lethargy, sedation, unconsciousness, and mortality, were meticulously documented and maintained in a distinct record. No signs of toxicity or mortality were observed after 14 days. Doses of 400 mg/kg for CUD and EAUD showed the most significant safety for anti-inflammatory activity.

Evaluation of anti-inflammatory activity

The rats were categorized into four groups, each comprising six rats. Paw edema was induced by administering 0.1 mL of 1% w/v carrageenan dissolved in 1% CMC and injected into the sub-plantar tissues of the left hind paw of each rat [54].

- Group I (Carrageenan control): 0.1 mL of 1% w/v carrageenan
- Group II (Positive control): Indomethacin 10 mg/kg b.w. + 0.1 mL of 1% w/v carrageenan
- Group III (Test I): CUD 400 mg/kg b.w. + 0.1 mL of 1% w/v carrageenan
- Group IV (Test II): EAUD 400 mg/kg b.w. + 0.1 mL of 1% w/v carrageenan.

The paw's thickness was measured using a Vernier Caliper just before and after the injection of carrageenan at 60, 120, 180, and 240 min. The percentage inhibition of edema in the test group compared to the carrageenan control group was used to calculate the anti-inflammatory activity.

The percentage inhibition of edema is calculated using the formula:

$$\% \text{ inhibition} = \frac{T_o - T_t}{T_o} \times 100$$

Where T_t is the thickness of the paw of the test group, and T_o is the paw thickness of the control group.

Molecular docking analysis

Molecular docking was performed using the phyto-constituents identified from GC-MS analysis of CUD and EAUD. The 3D structures of COX-2 (PDB ID: 3 mdl) [55], 5-LOX (PDB ID: 6 ncf) [56], PDE4 (PDB ID: 4 wcu) [57], and HP5 (PDB ID: 1 hd2) [58] were obtained from the Protein Data Bank. The 3D structure of Triamcinolone Acetonide (PubChem CID 6436), Campesterol (PubChem CID 173183), Docosanoic acid, 1,2,3-propanetriyl ester (PubChem CID 62726), 7,8-Epoxylanostan-11-ol, 3-acetoxy- (PubChem CID 541562), Anodendroside E 2 (PubChem CID 91692722), 7,8,12-Tri-O-acetyl-3-desoxy-ingol-3-one (PubChem CID 541413), 9-Octadecenoic acid (Z)-, 3-[(1-oxohexadecyl) oxy]-2-[(1-oxooctadecyl) oxy] propyl ester (PubChem CID 5365005), Epoxycholesterol (PubChem CID 227037), Holothurin acetate (PubChem CID 541418), Octadecanoic acid, 1-[[(1-oxohexadecyl) oxy] methyl]-1,2-ethanediyl ester (PubChem CID 91865316), Fluprednisolone (PubChem CID 5876), (±)9-HpODE (PubChem CID 6439847), (±)12(13)-DiHOME (PubChem CID 10236635), 2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-N-[2-(methylamino) ethyl]-1H-imidazo[4,5-c] pyridine-7-carboxamide (PubChem CID 25023715), 2,2-Dimethyl-4-[(octadecyloxy)methyl]-1,3-dioxolane (PubChem CID 568294), 2-Hydroxy-2-octyldecanedioic acid (PubChem CID 622473), 2-Nitro-4-nonanol (PubChem CID 544004), 3-(4-Methylphenyl) glutamic acid (GLU) (PubChem CID 319713), 3,4-Dihydro-2H-1,6-benzoxazocin-5(6H)-one (PubChem CID 550286), 6-Gingerol (PubChem CID 442793), Dihydro-1H,5H-dipyrrolo[1,2-a1',2'-d] pyrazine-2,5,7,10 (3H,8H,10aH)-tetrone (PubChem CID 544479), Ethephon (PubChem CID 27982), Ethyl 2-phenylethyl butylphosphonate (PubChem CID 6421933), Maltol (PubChem CID 8369), Methyl 3-[4-(2-methoxy-2-oxoethyl)-4-methyl-2,5-dioxo-3-pyrrolidinyl] propanoate (PubChem CID 597324), N-Benzylformamide (PubChem CID 80654), Phosphoglycolate (PubChem CID 529), TMB-8 (PubChem CID 5494), Traumatic Acid (PubChem CID 5283028), 2-Aminoadipic acid (PubChem CID 469), 2-Cyclohexyl-2-hydroxyacetamide (PubChem CID 236593), 4-Undecylbenzenesulfonic acid (PubChem CID 38222), 9-Oxo-10(E),12(E)-octadecadienoic acid (PubChem CID 5283011), Methyl 12-hydroxystearate (PubChem CID 8840), NP-008993 (PubChem CID 53851542), Oleamide (PubChem CID 5283387), Pyrogallol (PubChem CID 1057) were taken from PubChem. The proteins were designed with AutoDock tools-1.5.7, and the docking operation was carried out using the crystallographic structure chain A [59]. To determine the active site, the water molecules and co-crystal ligands were removed, followed by the addition of polar hydrogen, and then the Kollman charge was applied. The ligands were made using the Open Babel software (v.2.4.1) [60]. Active regions were found to be the parts of the amino acids that bind with the ligands shown in the protein files. To cover these areas, a grid box was made. For COX-2, 5-LOX, and PDE4, the grid box was set to have 60×60×60 x, y, and z points. For HP-5, it was set to 40×40×40. For COX-2, the grid center was set at 25.924, 21.223, and 65.974; for 5-LOX, it was 24.889, −22.451, and 33.681; for PDE4, it was 19.048, −11.068, and −4.778; and for HP-5, it was 8.009, 42.610, and 20.044. The docking score was determined using the AutoDock Vina software, and the 3D visualization was done using the Biovia Discovery Studio 2021 Client [61].

Statistical analysis

The statistical data were analyzed using a one-way analysis of variance followed by *post hoc* Tukey honestly significant difference test (Statistical Package for the Social Sciences v. 21, IBM corporation and MS EXCEL v. 2019), with value as mean+standard error mean. "p"<0.05 were considered statistically significant.

RESULTS

GC-MS analysis of chloroform and ethyl acetate of *U. distachya*

The GC-MS chromatograms of CUD and EAUD are illustrated in Fig. 1 and Table 2, representing the compounds' retention time, name of the compounds, nature of the compounds, molecular formula, molecular weight, peak area percentage, and biological activity. The structures of the phyto-compounds are illustrated in Fig. 2. The CUD possessed nine phytoconstituents, β Carotene (21.66); Triamcinolone Acetonide (2.97); Campesterol (1.83); Docisanoic Acid, 1,2,3-Propanetriyl ester (42.09); 7,8-Epoxylanostan-11-ol,3-acetoxy-(24.13); Anodendroside E 2 (0.24); Cholestan-3-ol,5-Chloro-6-Nitro-, (3 beta, 5 alpha, 6 beta)-(4.62); 7,8,12-Tri-O-Desoxy-ingol-3-one(0.63); 9-Octadecenoic Acid(Z)-, 3-[(1-Oxohexadecyl) Oxy]-2-[(1-Oxooctadecyl) Oxy] Propyl Ester (1.84). EAUD were reveled seven phytoconstituent, Epoxycholesterol (15.56); 9-Octadecenoic acid (Z)-, 3-[(1-oxohexadecyl)oxy]-2-[(1-oxooctadecyl)oxy] propyl ester (3.39); Holothurin acetate (7.85); Octadecanoic acid, 1-[[(1-oxohexadecyl)oxy]methyl]-1, 2-ethanediyl ester (26.08); Fluprednisolone (12.73); 7,8-Epoxylanosta-11-ol, 3-acetoxy-(21.15); Cholestan-3-ol, 5-chloro-6-nitro-, (3 bera, 5 alpha, 6 beta)-(13.25).

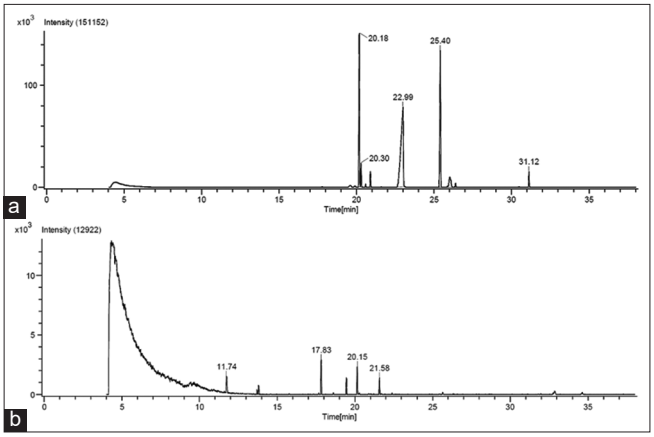

Fig. 1: Gas chromatography-mass spectrometry chromatogram of (a) chloroform, and (b) ethyl extract of *Urochloa distachya*

LC-MS analysis of chloroform and ethyl acetate of *U. distachya*

The LC-MS/MS chromatograms of CUD and EAUD are illustrated in Figs. 3 and 5, and the structure of the compounds is depicted in Figs 4 and 6. Tables 3 and 4 represent the peak number, retention time, identified compounds, ion, molecular formula, molecular weight, and biological activity. The positive ion mode (M+H)+of the CUD indicated the probable compounds as such; Maltol; 2-Nitro-4-Nonanol; 3,4-Dihydro-2H-1,6-Benzoxazocin-5(6H)-One; N-Benzylformamide; Methyl 3-[4-(2-Methoxy-2-Oxoethyl)-4-Methyl-2,5-Dioxo-3-Pyrrolidinyl] Propanoate; 2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-N-[2-(methylamino) ethyl]-1H-imidazo[4,5-c]pyridine-7-carboxamide; (3Z)-3-{4-[2-(4 Methoxyphenyl)-2-oxoethoxy] benzylidene}-6-methyl-2,3-dihydro-4H-thiochromen-4-one; 6-Gingerol; (±) 12(13)-DiHOME; TMB-8; 1,3-Diacetoxy-7,8-dimethyl-7-[(2E)-3-methyl-2,4-pentadiene-1-yl]-3,5,6,6a,7,8,9,10-octahydronaphtho[1,8a-c] furan-5-yl4-hydroxybenzoate; Dilauryl Methylamine; Hexyl 2-propylheptyl phthalate; Ethephon; Phosphoglycolate. Negative ion mode [M-H]-identified the compounds in *U. distachya* chloroform extract; D-(-)- Mannitol; 3-(4-Methylphenyl) GLU; Dihydro-1H,5H-dipyrrolo[1,2-a:1',2'-d]pyrazine-2,5,7,10(3H,8H,10aH)-tetrone; NP-014937; Traumatic Acid; 2-Hydroxy-2-octyldecanedioic acid; Ethyl 2-phenylethyl butylphosphonate; (±) 9-HpODE; Tetradecyl N-(ethoxycarbonyl)alaninate; Glycol stearate; 2,2-Dimethyl-4-[(octadecyloxy)methyl]-1,3-dioxolane. The positive ion mode (M+H)+ of the ethyl acetate extract of *U. distachya* showed compounds such as; 2,4,5,6-Tetrachloro nicotinamide; 2-Acetamido-1,5-anhydro-2-deoxyhex-1-enitol; 2-Cyclohexyl-2-hydroxyacetamide; Pyrogallol; 1-Allyl-2-benzyl-1,2-dihydropyridine; (4aR,4bR,8S,10aR,10bR,12aS)-8-Hydroxy-10a,12a-dimethyl-3,4,4a,4b,5,7,8,9,10,10a,10b,11,12,12a-tetradecahydronaphtho[2,1-f]quinolin-2(1H)-one; 4-[5-Acetoxy-8,8-dimethyl-6-(3-methyl-2-buten-1-yl)-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl]-1,2-phenylene diacetate; NP-008993; Oleamide; 9-Oxo-10(E),12(E)-octadecadienoic acid; 2-Methoxy-

Table 2: GC-MS study of CUD and EAUD

Sl. No.	Rt (min)	Name of the compound	Nature	M.F.	M.W.	Peak area %	Biological activity	References
01	20.18	β Carotene	Polyunsaturated hydrocarbon	$C_{40}H_{56}$	536.9	21.66	Antibacterial, Antioxidant, and Antidiabetic activity.	[62,63]
02	20.30	Triamcinolone Acetonide	Corticosteroid	$C_{24}H_{31}FO_6$	434	2.97	Anti-inflammatory, analgesic, rheumatoid arthritis, Addison's disease, leukemia, osteoarthritis, and hypersensitivity. Spasticity. Itching, redness, inflammation, severe allergic reactions, severe colitis, tendons, lung disorders, blood cell disorders, inflammation, and eye disorders.	[64-66]
03	20.90	Campesterol	Steroidal compound	$C_{28}H_{48}O$	400	1.83	Anti-inflammatory and cytotoxic activity. Antioxidant and anticancer. Antibacterial.	[48,67,68]
04	22.99	Docosanoic acid, 1,2,3-propanetriyl ester	Fatty acid ester	$C_{69}H_{134}O_6$	1058	42.09	Emulsifying agent, skin conditioning agent, and surfactant. Antioxidant, nematicide, and hypocholesterolemic activity.	[69,70]
05	25.40	7,8-Epoxylanostan-11-ol, 3-acetoxy-	Alcoholic	$C_{32}H_{54}O_4$	502	24.13	Anti-inflammatory, antimicrobial activity.	[71,72]
06	25.86	Anodendroside E 2	NA	$C_{30}H_{38}O_{11}$	574	0.24	Antimicrobial.	[73]
07	26.02	Cholestan-3-ol, 5-Chloro-6-Nitro-, (3 beta, 5 alpha, 6 beta) -	NA	$C_{27}H_{46}ClNO_3$	468.1	4.62	Not Reported	
08	26.39	7,8,12-Tri-O-acetyl-3-desoxy-ingol-3-one	NA	$C_{26}H_{34}O_9$	490	0.63	Antioxidant and antibacterial activity.	[74]
09	31.12	9-Octadecenoic acid (Z)-, 3-[(1-oxohexadecyl) oxy]-2-[(1-oxooctadecyl) oxy] propyl ester	NA	$C_{55}H_{104}O_6$	860	1.84	Emulsifying agent.	[75]
10	11.74	Epoxycholesterol	Steroid	$C_{27}H_{46}O_2$	402.7	15.56	Decreases ABCA1-and ABCG1-medicated cholesterol efflux. Marked pro-inflammatory and cytotoxic effects.	[76,77]
11	13.70	9-Octadecenoic acid (Z)-, 3-[(1-oxohexadecyl) oxy]-2-[(1-oxooctadecyl) oxy] propyl ester	Long chai fatty acid	$C_{55}H_{104}O_6$	860	3.39	Emulsifying.	[75]
12	13.80	Holothurin acetate	Triterpene glycoside	$C_{33}H_{48}O_7$	556	7.85	Antischistosomal agent.	[78]
13	17.83	Octadecanoic acid, 1-[[(1-oxohexadecyl) oxy] methyl]-1,2-ethanediylester	Long-chain fatty acid	$C_{55}H_{106}O_6$	863.4	26.08	Drug synthesis-Osteosarcoma.	[79]
14	19.45	Fluprednisolone	Fluorinated Steroid	$C_{21}H_{27}FO_5$	378	12.73	Anti-inflammatory activity.	[80]
15	20.15	7,8-Epoxylanostan-11-ol, 3-acetoxy-	Alcoholic compound	$C_{32}H_{54}O_4$	502	21.15	Anti-inflammatory and antimicrobial activity.	[81,82]
16	21.58	Cholestan-3-ol, 5-Chloro-6-Nitro-, (3 beta, 5 alpha, 6 beta)-	NA	$C_{27}H_{46}ClNO_3$	468.1	13.25	Not Reported	

GC-MS: Gas chromatography-mass spectrometry, CUD: chloroform extract of *Urochloa distachya*, EAUD: Ethyl acetate extracts of *Urochloa distachya*

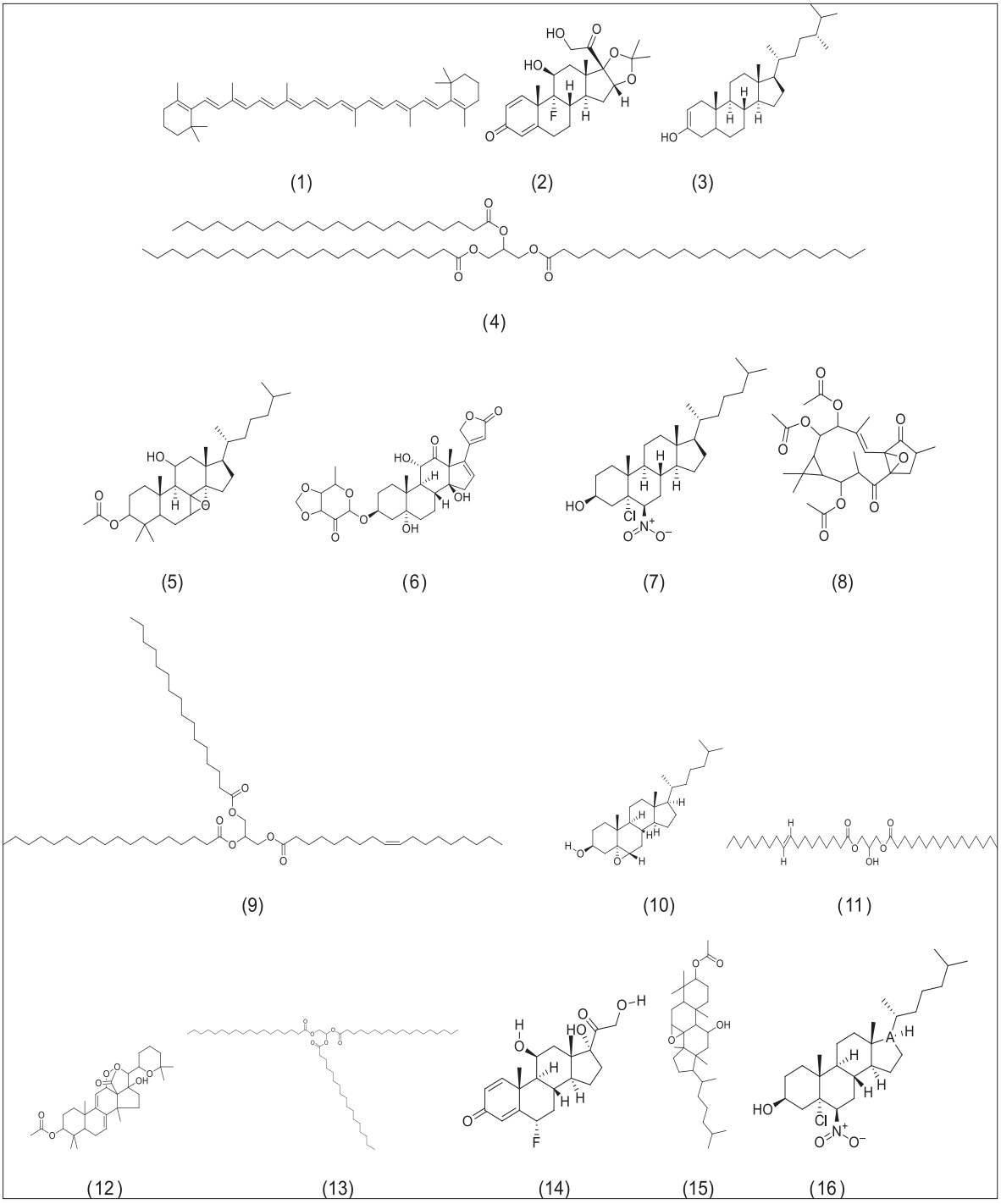

Fig. 2: Structure of the compounds from gas chromatography-mass spectrometry analysis of chloroform and ethyl acetate extract of *Urochloa distachya*

N,N-diundecylacetamide; 2-Aminoadipic acid; Hexyl2-propylheptyl phthalate; N,N'-ethylenebis palmitamide; N-2-Propyn-1-ylcysteine. Negative ion mode (M-H)- found the compounds in the ethyl acetate extract of *U. distachya* were; D-(-)-Mannitol; 3-hydroxy-2-{[(2E)-3-(4-hydroxyphenyl)prop-2-enoyl]oxy}-3-(methoxycarbonyl) pentanedioic acid; 4-Undecylbenzenesulfonic acid; Tetradecyl N-(ethoxycarbonyl) alaninate; Glycol stearate; Methyl 12-hydroxystearate; NP-021293.

Anti-inflammatory activity

Table 5 and Fig. 7 represent the graph of change in paw edema. Fig. 8 compares the effects of CUD and EAUD extracts and the conventional drug on the carrageenan control during different intervals in a carrageenan-induced paw edema model using a Vernier Caliper. Administration of CUD at a dosage of 400 mg/kg body weight orally inhibited carrageenan-induced paw edema, exhibiting percentage inhibitions of 22.89%, 31.80%, 57.89%, and 57.9% at 1-4 h. Similarly, the administration of EAUD exhibited percentage inhibitions of 5.38%, 5.64%, 31.73%, and 26.5%, respectively. Indomethacin administered at 10 mg/kg orally inhibited carrageenan-induced paw edema by 43.09%, 50.32%, 75.15%, and 83.7% at 1, 2, 3, and 4 h, respectively.

Table 3: LC-MS analysis of positive (M+H)+ and negative (M-H)- ion mode of CUD

Sl. No.	Name of the compound	Nature	M.F.	M.W.	Biological activity	References
1	Maltol	Organic compound	$C_6H_6O_3$	126.03	Antimicrobial activity, natural preservative for cosmetic formulation. Anti-inflammatory activity. Anticancer activity. Antioxidant activity.	[83,84]
2	2-Nitro-4-nonanol	-	$C_9H_{19}NO_3$	189.14	Not reported	
3	3,4-Dihydro-2H-1,6-benzoxazocin-5 (6H)-one	Benzoxazine	$C_{10}H_{11}NO_2$	177.08	Not reported	
4	N-Benzylformamide	Aromatic compound	C_8H_9NO	135.06	Not reported	
5	Methyl 3-[4-(2-methoxy-2-oxoethyl)-4-methyl-2,5-dioxo-3-pyrrolidinyl] propanoate	-	$C_{12}H_{17}NO_6$	271.10	Not reported	
6	2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-N-[2-(methylamino) ethyl]-1H imidazo[4,5-c] pyridine-7-carboxamide	-	$C_{14}H_{18}N_8O_2$	330.16	Not reported	
7	(3Z)-3-{4-[2-(4-Methoxyphenyl)-2-oxoethoxy] benzylidene}-6-methyl-2,3-dihydro-4H-thiochromen-4-one	-	$C_{26}H_{22}O_4S$	430.12	Not reported	
8	6-Gingerol	Phenolic	$C_{17}H_{26}O_4$	294.18	Anticancer, anti-inflammatory, antioxidant activity.	[85]
9	(±) 12 (13)-DiHOME		$C_{18}H_{34}O_4$	314.24		
10	TMB-8	Trihydroxybenzoic acid	$C_{22}H_{37}NO_5$	395.27	Ca2+antagonist	[86]
11	1,3-Diacetoxy-7,8-Dimethyl-7-[(2E)-3-Methyl-2,4-Pentadien-1-yl]-3,5,6,6a, 7,8,9,10-Octahydronaphtho[1,8a-c] Furan-5-yl4-Hydroxybenzoate	-	$C_{31}H_{38}O_8$	538.26	Not reported	
12	Dilauryl Methylamine	-	$C_{25}H_{53}N$	367.42	Not reported	
13	Hexyl 2-propylheptyl phthalate	Organic compound	$C_{24}H_{38}O_4$	390.28	Not reported	
14	Ethephon	Phosphonic acid	$C_2H_6ClO_3P$	143.97	Fruit and vegetable ripener	[87]
15	Phosphoglycolate	Monocarboxylic acid	$C_2H_5O_6P$	155.98	Not reported	
16	D-(-)-Mannitol	Sugar alcohol	$C_6H_4N_2OP_2$	181.98	Cerebral edema, decreased intra-ocular pressure, diuresis, reduced renal cell swelling.	[88]
17	3-(4-Methylphenyl) glutamic acid	Amino acid	$C_{12}H_{15}NO_4$	237.10	Not reported	
18	Dihydro-1H,5H-dipyrrolo[1,2-a: 1',2'-d] pyrazine-2,5,7,10 (3H,8H,10aH)-tetrone	-	$C_{10}H_{10}N_2O_4$	222.06	Not reported	
19	NP-014937	-	$C_{25}H_{26}O_{13}$	534.14	Not reported	
20	Traumatic Acid	Monounsaturated ted straight-chain dicarboxylic acid, Plant hormone	$C_{12}H_{20}O_4$	228.14	Antioxidant activity, arterial hypertension, skin diseases.	[89]
21	2-Hydroxy-2-octyldecanedioic acid	Hydroxy fatty acid	$C_{18}H_{34}O_5$	330.24	Note reported	
22	Ethyl2-phenylethyl butylphosphonate	Phosphonic ester	$C_{14}H_{23}O_3P$	270.14	Not reported	
23	(±) 9-HpODE	Hydroperoxy polyunsaturated fatty acid	$C_{18}H_{32}O_4$	312.23	Antimicrobial activity	[90]
24	Tetradecyl N-(ethoxycarbonyl) alaninate	-	$C_{20}H_{39}NO_4$	357.29	Not reported	
25	Glycol stearate	Organic compound	$C_{20}H_{40}O_3$	328.30	Emulsifying agent.	[91]
26	2,2-Dimethyl-4-[(octadecyloxy) methyl]-1,3-dioxolane	-	$C_{24}H_{48}O_3$	384.36	Not reported	

LC-MS: Liquid chromatography-mass spectrometry, CUD: chloroform extract of *Urochloa distachya*

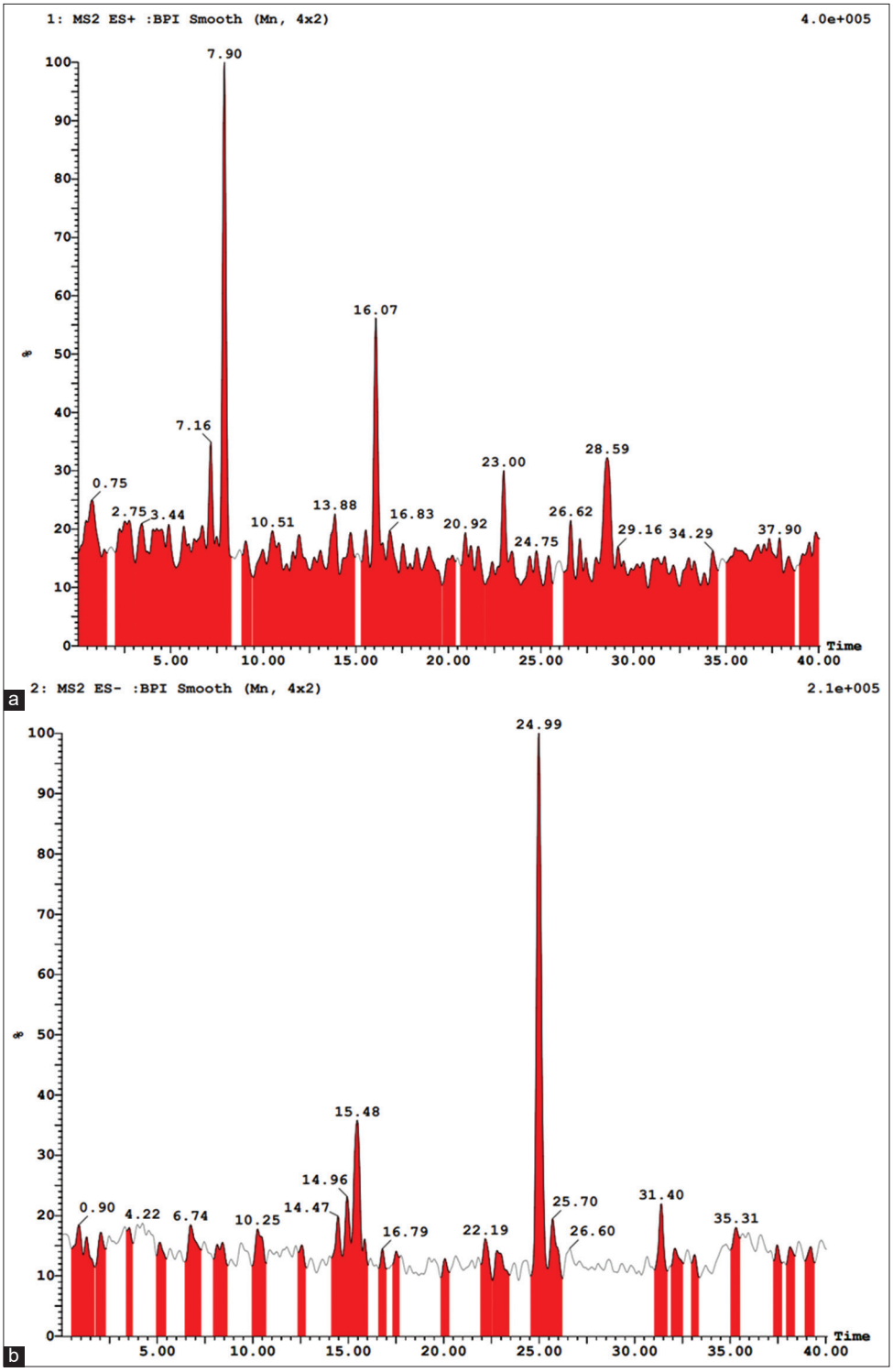

Fig. 3: Liquid chromatography-mass spectrometry data (a) positive and (b) negative ion mode of chloroform extract of *Urochloa distachya*

Molecular docking

Molecular docking studies were conducted to investigate the anti-inflammatory activities of compounds from CUD and EAUD, which were identified by GC-MS and LC-MS analysis. In this investigation, 38 compounds were docked into the binding sites of COX-2 (3 mdl), 5-LOX (6 ncf), PDE4 (4 wcu), and HP5 (1 hd2). The docking score revealed that all ligands showed activity ranging from −4.3 to −15.3 against COX-2, −4.6−−13.6 against 5-LOX, −3.9−−17.1 against PDE4, and −3.9−−11.6 against HP5 (Tables 6 and 7). According to their overall docking score, the drugs exhibited relatively superior binding efficacy against COX-2 and PDE4 compared to two other target receptors. The docking score, hydrogen bond interacting residues, and distance between the interaction of ligands are given in Tables 6 and 7, and the 2D and 3D structures of interacted molecules are illustrated in Figs. 9-14.

DISCUSSION

The GC-MS and LC-MS investigations of CUD and EAUD identified a variety of bioactive compounds that provide strong evidence for the observed anti-inflammatory and other therapeutic activities in the

Table 4: LC-MS analysis of positive (M+H)+, and negative (M-H)- ion mode of EAUD

Sl. No.	Name of the compound	Nature	M.F.	M.W.	Biological activity	References
1	2,4,5,6-Tetrachloro nicotinamide	Vitamin	$C_6H_2Cl_4N_2O$	257.89	Not reported	
2	2-Acetamido-1,5-anhydro-2-deoxyhex-1-enitol	-	$C_8H_{13}NO_5$	203.08	Not reported	
3	2-Cyclohexyl-2-hydroxyacetamide	-	$C_8H_{15}NO_2$	157.11	Not reported	
4	Pyrogallol	Phenolic	$C_6H_6O_3$	126.03	Antibacterial activity. Inhibitor for anticholinesterase.	[92,93]
5	1-Allyl-2-benzyl-1,2-dihydropyridine	-	$C_{15}H_{17}N$	211.14	Not reported	
6	(4aR,4bR,8S,10aR,10bR,12aS)-8-Hydroxy-10a, 12a-dimethyl-3,4,4a, 4b, 5,7,8,9,10,10a, 10b, 11,12,12a-tetradecahydronaphtho[2,1-f] quinolin-2 (1H)-one	-	$C_{19}H_{29}NO_2$	303.22	Not reported	
7	4-[5-Acetoxy-8,8-dimethyl-6-(3-methyl-2-buten-1-yl)-4-oxo-4H,8H-pyrano[2,3-f] chromen-3-yl]-1,2-phenylene diacetate	-	$C_{31}H_{30}O_9$	546.19	Not reported	
8	NP-008993	Fatty acid	$C_{20}H_{40}O_5$	360.29	Not reported	
9	Oleamide		$C_{18}H_{35}NO$	281.27		
10	9-Oxo-10(E),12(E)-octadecadienoic acid	Natural agonist	$C_{18}H_{30}O_3$	294.22	Inhibit cellular triglyceride in the hepatocytes.	[94]
11	2-Methoxy-N, N-diundecylacetamide	-	$C_{25}H_{51}NO_2$	397.39	Not reported	
12	2-Aminoadipic acid	Alpha-amino acid	$C_6H_{11}NO_4$	161.07	Anti-inflammatory, neuroprotective activity.	[95]
13	Hexyl 2-propylheptyl phthalate	Organic compound	$C_{24}H_{38}O_4$	390.28	Not reported	
14	N, N'-ethylenebis palmitamide	Organic compound	$C_{34}H_{68}N_2O_2$	536.53	Not reported	
15	N-2-Propyn-1-ylcysteine	Organic compound	$C_6H_9NO_2S$	159.03	Not reported	
16	D-(-)-Mannitol	Sugar alcohol	$C_6H_4N_2OP_2$	181.98	Cerebral edema, decreased intra-ocular pressure, diuresis, reduced renal cell swelling.	[88]
17	3-hydroxy-2-{[(2E)-3-(4-hydroxyphenyl) prop-2-enoyl] oxy}-3-(methoxycarbonyl) pentanedioic acid	-	$C_{28}H_{24}N_{10}O_4$	564.20	Not reported	
18	4-Undecylbenzenesulfonic acid	Arenesulfonic acid	$C_{17}H_{28}O_3S$	312.18	Not reported	
19	Tetradecyl N-(ethoxycarbonyl) alaninate	-	$C_{20}H_{39}NO_4$	357.29	Note reported	
20	Glycol stearate	Organic compound	$C_{20}H_{40}O_3$	328.30	Emulsifying agent.	[91]
21	Methyl 12-hydroxystearate	Fatty acid	$C_{19}H_{38}O_3$	314.28	Textile ancillary, cosmetics, and paper industry	[96]
22	NP-021293	-	$C_5H_9O_6PS$	259.92	Note reported	

LC-MS: Liquid chromatography-mass spectrometry, EAUD: Ethyl acetate extracts of *Urochloa distachya*

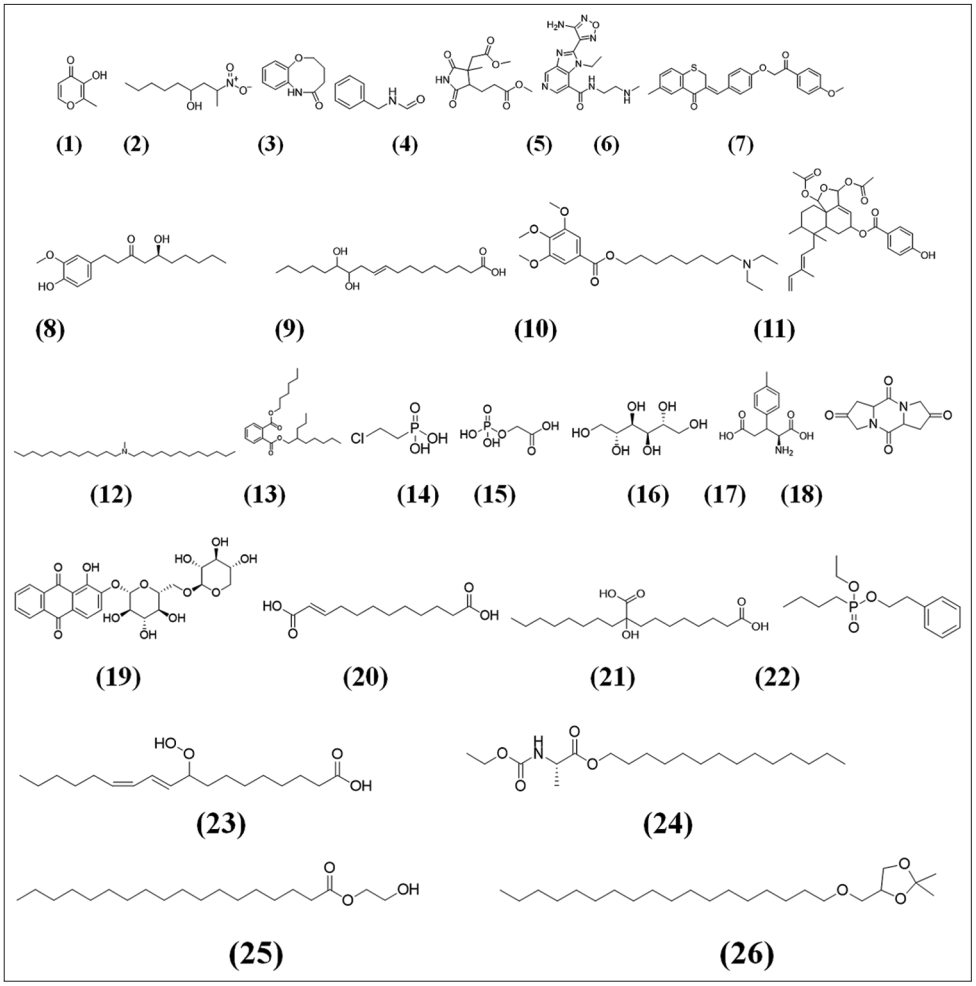

Fig. 4: Structure of the compounds from liquid chromatography-mass spectrometry analysis of positive (M+H)+and negative (M-H)- ion mode of chloroform extract of *Urochloa distachya*

Table 5: *In vivo* anti-inflammatory activity

Group	Treatment	Change in paw thickness (mm)±SD (% inhibition)			
		1st h	2nd h	3rd h	4th h
Group I: Carrageenan Control	Carrageenan induced (0.1 mL of 1% w/v)	2.17±0.12	2.60±0.14	3.42±0.12	3.07±0.10
Group II: Positive Control	Carrageenan induced (0.1 mL of 1% w/v)+Indomethacin 10 mg/kg b.w.	1.24±0.03a* 43.09%	1.29±0.01a* 50.32%	0.85±0.15a* 75.15%	0.50±0.13a* 83.7%
Group III: Test-1	Carrageenan induced (0.1 mL of 1% w/v)+CUD 400 mg/kg b.w.	1.67±0.02b* 22.89%	1.77±0.01b* 31.80%	1.44±0.02b* 57.89%	1.29±0.02b* 57.9%
Group IV Test-2	Carrageenan induced (0.1 mL of 1% w/v)+EAUD 400 mg/kg b.w.	2.05±0.03c* 5.38%	2.45±0.03c* 5.64%	2.34±0.02c* 31.73%	2.26±0.03c* 26.5%

The data are expressed as+standard error mean (n=6). The values were carried out by One-way analysis of variance (Tukey's test). a*, b*, and c* = p<0.05 compared to carrageenan control group. CUD: Chloroform extract of *Urochloa distachya*, EAUD: Ethyl acetate extract of *Urochloa distachya*

study. Triamcinolone Acetonide, a corticosteroid identified in the GC-MS analysis, is known for its wide range of therapeutic effects, including anti-inflammatory, analgesic, and immunosuppressive activities, making it useful for conditions such as rheumatoid arthritis, Addison's disease, leukemia, and various allergic reactions [64-66]. Campesterol, another steroidal compound, exhibited not only anti-inflammatory effects but also cytotoxic, antioxidant, anticancer, and antibacterial properties, highlighting its therapeutic versatility [48,67,68]. The alcoholic compound 7,8-Epoxylanostan-11-ol, 3-acetoxy-, showed anti-inflammatory and antimicrobial activities [71,72], whereas Epoxycholesterol demonstrated pro-inflammatory and cytotoxic effects, as well as a role in cholesterol efflux regulation [76,77]. Fluprednisolone, a steroid, is well-established for its anti-inflammatory activity, contributing to the study's findings [80].

The LC-MS analysis revealed Maltol, an organic compound with a broad spectrum of biological activities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer effects, making it valuable for cosmetic and therapeutic applications [83,84,97,98]. 6-Gingerol, a phenolic compound, showed potent anti-inflammatory, antioxidant, and anticancer properties [85], whereas 2-Aminoadipic acid, an alpha-amino acid, exhibited anti-inflammatory and neuroprotective activities. Collectively, these compounds considerably improve the study's findings on the anti-inflammatory potential of CUD

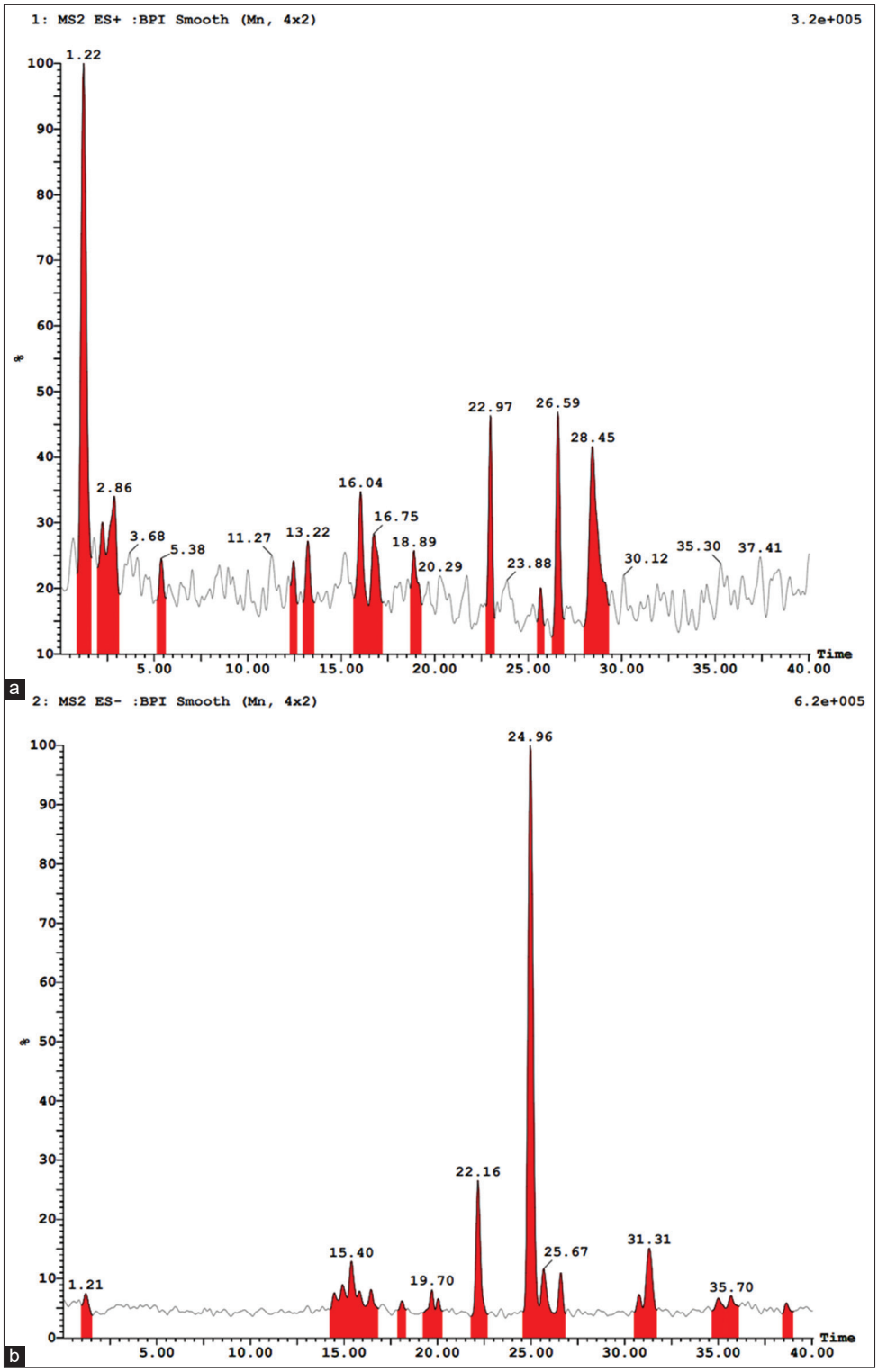

Fig. 5: Liquid chromatography-mass spectrometry data (a) positive and (b) negative ion mode of ethyl acetate extracts of *Urochloa distachya*

and EAUD, thereby further supporting their diverse therapeutic applications.

Inflammation is a complex process that results from a severe disruption in tissue homeostasis [99]. The paw edema resulting from carrageenan is a biphasic phenomenon. The initial phase, occurring within the 1st h post-injection, is driven by the release of histamine, serotonin, and kinins. The second phase, beginning at the end of the first and lasting 2-3 h, is predominantly mediated by prostaglandin [100]. The *in vivo* anti-inflammatory potential of both CUD and EAUD was assessed using the carrageenan-induced paw edema model. The findings showed that CUD had better anti-inflammatory properties relative to EAUD. The CUD-treated group exhibited a notable decrease in paw edema, especially in the later stages of inflammation, indicating that CUD effectively suppressed both the initial and later phases of the inflammatory response. On the other hand, EAUD exhibited anti-inflammatory

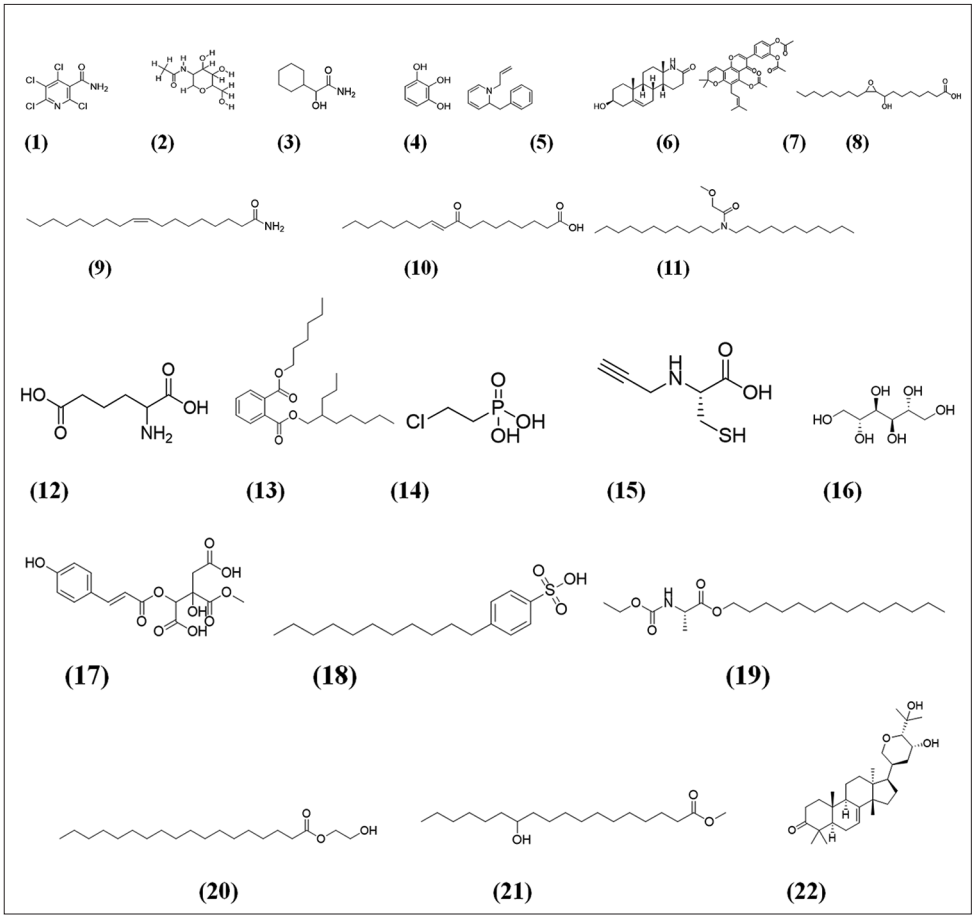

Fig. 6: Structure of the compounds from liquid chromatography-mass spectrometry analysis of positive (M+H)+, and negative (M-H)- ion mode of ethyl acetate extracts of *Urochloa distachya*

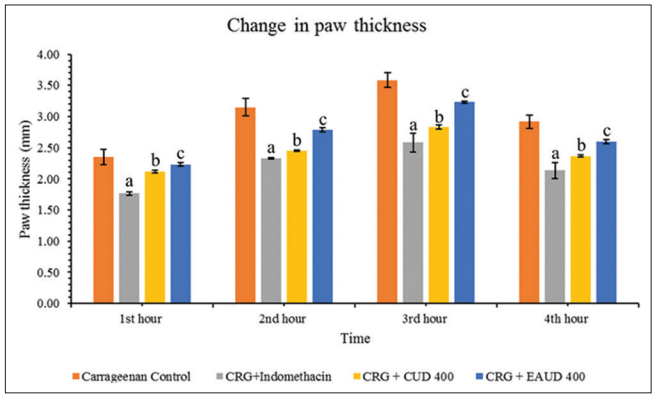

Fig. 7: Change in paw edema by 400 mg/kg b.w. of chloroform extract of *Urochloa distachya* and ethyl acetate extracts of *U. distachya* in rats induced with carrageenan

activity but yielded a comparatively lesser response, indicating that the compounds found in CUD may possess a more reliable or sustained effect in mitigating inflammation. The findings indicate that CUD possesses significant potential as an anti-inflammatory effect.

Anodendroside E 2, Triamcinolone Acetonide, Fluprednisolone, Maltol, 6-Gingerol, and 2-Aminoadipic acid were identified through GC-MS and LC-MS analyses of the CUD and EAUD, demonstrating notable anti-inflammatory activity. In this study, molecular docking analysis was performed to evaluate the binding capacity of these compounds with inflammatory targets such as COX-2, 5-LOX, PDE4, and HP5.

A hydrogen bond forms between the nitrogen atom of the imidazole side chain of histidine (HIS)351 and the oxygen atom of Anodendroside E 2. This interaction stabilizes the compound at the COX-2 active site. The hydrogen bond forms between the amide side chain of glutamine (GLN)565 and a hydroxyl group on Anodendroside E 2, enhancing its binding affinity. Two hydrogen bonds are established between the primary nitrogen atoms of glycine (GLY)354 and two oxygen atoms of Anodendroside E 2, highlighting the dual interaction locations with this residue. The hydroxyl group of threonine (THR)94 establishes a hydrogen bond with an oxygen atom in the molecule, enhancing the stability of the contact within the binding pocket. The oxygen atoms (O) of hydroxyl (-OH), ester, or carbonyl groups in Anodendroside E 2 are probably engaging in hydrogen bond formation with the hydrogen atoms of the amino acid side chains (Serine [SER]171, GLN611, GLU622) in the enzyme 5-LOX. In the interaction between Anodendroside E 2 and PDE4. Asparagine (ASN)209 forms two hydrogen bonds with Anodendroside E 2 at distances of 2.03 Å and 2.22 Å, likely involving the oxygen atoms of the hydroxyl or carbonyl groups on the ligand. These short distances indicate strong hydrogen bonds. GLN210 forms a weaker hydrogen bond at a distance of 3.22 Å, likely interacting with an oxygen atom of the ligand's hydroxyl or ester groups. Aspartic acid (ASP)318 also forms a weaker hydrogen bond at a distance of 3.39 Å, probably involving its carboxyl group interacting with an oxygen atom from Anodendroside E 2. In the interaction between Anodendroside E 2 and HP-5, two key residues are involved in hydrogen bonding. ASN21 forms a hydrogen bond with an oxygen atom of Anodendroside E 2 at a distance of 2.12 Å, indicating a strong interaction. GLU16 also forms a hydrogen bond at a slightly longer distance of 2.76 Å, likely involving the hydrogen atom attached to the hydroxyl group of Anodendroside E 2. The 3D and 2D interactions are illustrated in Fig. 9.

Fig. 8: Effect of carrageenan, indomethacin, chloroform extract of *Urochloa distachya* (CUD), and ethyl acetate extracts of *U. distachya* (EAUD) on the left hind paw of rats at 1 h and 4 h. (1a and b) showed the effect of carrageenan induced (0.1 mL of 1% w/v). (2a and b) showed the effect of indomethacin 10 mg/kg b.w. (3a and b) showed the effect of carrageenan induced (0.1 mL of 1% w/v)+CUD 400 mg/kg b.w. (4a and b) showed the effect of carrageenan induced (0.1 mL of 1% w/v)+EAUD 400 mg/kg b.w

Table 6: Binding energies (Kcal/mol) of phytochemicals identified through GC-MS and LCMS analysis of CUD and EAUD assessed for anti-inflammatory potential via molecular docking studies

Sl. No	Name of the compound	COX-2 (3 mdl)	5-LOX (6 ncf)	PDE4 (4 wcu)	HP5 (1 hd2)
1	Indomethacin	−6.7	−7.1	−9.6	−6.5
2	Triamcinolone Acetonide	−8.3	−8.2	−9.5	−6.4
3	Campesterol	−7.4	−7.5	−8.7	−6.9
4	Docosanoic acid, 1,2,3-propanetriyl ester	−5.7	−3.1	−6.3	−3.5
5	7,8-Epoxylanostan-11-ol, 3-acetoxy-	−8.6	−7.8	−7.9	−6.7
6	Anodendroside E 2	−10.3	−9.3	−10.7	−7.9
7	7,8,12-Tri-O-acetyl-3-desoxy-ingol-3-one	−11.6	−9.9	−12.5	−8.6
8	9-Octadecenoic acid (Z)-, 3-[(1-oxohexadecyl) oxy]-2-[(1-oxooctadecyl) oxy] propyl ester	−5.1	−5.2	−6.3	−3.4
9	Epoxycholesterol	−7.3	−6.9	−8.7	−6.4
10	Holothurin acetate	−15.3	−13.6	−17.1	−11.6
11	Octadecanoic acid, 1-[[(1-oxohexadecyl) oxy] methyl]-1,2-ethanediyl ester	−6.5	−6.3	−6.5	−3.1
12	Fluprednisolone	−7.9	−7.3	−9.5	−6.5
13	(±) 9-HpODE	−6.9	−6.2	−6.7	−5.2
14	(±) 12 (13)-DiHOME	−6.9	−6.2	−5.9	−5.2
15	2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-N-[2-(methylamino) ethyl]-1H-imidazo[4,5-c] pyridine-7-carboxamide	−7.2	−7.1	−7.3	−6.4
16	2,2-Dimethyl-4-[(octadecyloxy) methyl]-1,3-dioxolane	−6.7	−7.4	−7.5	−5.4
17	2-Hydroxy-2-octyldecanedioic acid	−6.9	−7.7	−7.5	−5.6
18	2-Nitro-4-nonanol	−6.1	−5.6	−5.6	−4.5
19	3-(4-Methylphenyl) glutamic acid	−6.3	−6.4	−6.9	−5.7
20	3,4-Dihydro-2H-1,6-benzoxazocin-5 (6H)-one	−7.4	−7.5	−6.9	−5.6
21	6-Gingerol	−6.7	−6.5	−6.9	−5.2
22	Dihydro-1H,5H-dipyrrolo[1,2-a1’,2’-d] pyrazine-2,5,7,10 (3H,8H,10aH)-tetrone	−7.7	−6.6	−7.3	−5.7
23	Ethephon	−4.3	−4.6	−3.9	−3.9
24	Ethyl 2-phenylethyl butylphosphonate	−7.1	−7.3	−6.3	−4.6
25	Maltol	−5.7	−5.7	−5.4	−4.7
26	Methyl 3-[4-(2-methoxy-2-oxoethyl)-4-methyl-2,5-dioxo-3-pyrrolidinyl] propanoate	−5.9	−5.9	−6.5	−5.5
27	N-Benzylformamide	−6.2	−6.1	−6.6	−4.7
28	Phosphoglycolate	−5.0	−4.8	−4.8	−4.6
29	TMB-8	−7.3	−7.3	−7.3	−6.3
30	Traumatic Acid	−6.3	−4.9	−5.8	−5.3
31	2-Aminoadipic acid	−5.7	−5.2	−5.5	−4.7
32	2-Cyclohexyl-2-hydroxyacetamide	−6.9	−5.8	−6.6	−5.2
33	4-Undecylbenzenesulfonic acid	−6.5	−4.9	−6.7	−5.4
34	9-Oxo-10(E),12(E)-octadecadienoic acid	−6.3	−6.4	−6.7	−5.3
35	Methyl 12-hydroxystearate	−7.1	−6.5	−6.5	−5.0
36	NP-008993	−6.5	−5.9	−−6.5	−5.4
37	Oleamide	−6.8	−5.8	−6.0	−4.7
38	Pyrogallol	−5.8	−5.4	−5.3	−5.3

LC-MS: Liquid chromatography-mass spectrometry, EAUD: Ethyl acetate extracts of *Urochloa distachya*, CUD: chloroform extract of *Urochloa distachya*

Table 7: Hydrogen bond, amino acid residue, and interaction distance of phyto-compounds from GC-MS analysis of CUD and EAUD

Sl. No.	Name of the compound	COX-2 (3 mdl)		5-LOX (6 ncf)		PDE4 (4 wcu)		HP5 (1 hd2)	
		Hydrogen bonds	Distance (Å)	Hydrogen bonds	Distance (Å)	Hydrogen bonds	Distance (Å)	Hydrogen bonds	Distance (Å)
1	Indomethacin	HIS351, LYS358, ASP347, GLN350	1.77, 2.19, 2.72, 3.99	GLU16, ASP170	2.14, 3.73	SER368	3.29	GLU91, GLY92, LYS93, GLY82	2.34, 1.92, 2.78, 2.22
2	Anodendroside E 2	HIS351, GLN565, GLY354, GLY354, THR94	1.95, 1.85, 3.49, 3.67, 3.26	SER171, GLN611, GLU622	2.62, 2.47, 2.46	ASN209, ASN209, ASP318, GLN210	2.03, 2.22, 3.39, 3.22	ASN21, GLU16	2.12, 2.76
3	Campesterol	-	-	LYS83	3.46	-	-	GLU83	2.06
4	Triamcinolone Acetonide	HIS351, GLN565, GLN350, GLY354, SER579	2.67, 2.48, 2.22, 3.41, 3.50	ARG401, ASN613	2.82, 2.07	HIS160, HIS204, ASN209, THR271, GLU230, HIS160	2.72, 2.30, 2.82, 2.38, 2.51, 3.56	GLU91, ARG95	2.43, 3.63
5	Epoxycholesterol	PRO191	3.00	-	-	HIS160, ASP318, HIS200	2.50, 2.68, 3.64	-	-
6	Fluprednisolone	HIS356, GLN565, HIS356, ASP347	3.56, 2.38, 3.60, 3.56	ASP170	2.59	HIS204, VAL207	2.73, 2.62	GLY92, ARG95, GLY85, GLU91	2.19, 2.65, 3.50, 3.28
7	(±) 9-HpODE	MET522, VAL523	2.32, 2.95	TYR470, GLN549, LEU448	3.07, 2.33, 2.82	THR333, HIS164, HIS200, ASP318	3.12, 3.06, 2.92, 2.04	ASN21, ARG86, ALA90, VAL94, GLY17, GLY17, ARG95	2.32, 3.08, 2.32, 2.35, 3.50, 3.62, 3.46
8	(±) 12 (13)-DiHOME	-	-	TYR81, TYR100	2.60, 2.95	TYR159	3.36	LYS63, LYS63, LYS63, LEU96, GLU16, VAL70	1.95, 2.46, 2.46, 1.89, 2.54, 2.91
9	2-(4-Amino-1,2,5-oxadiazol -3-yl)-1-ethyl-N-[2-(methylamino) ethyl]-1H-imidazo[4,5-c] pyridine-7-carboxamide	ASN537, GLY225, ASN375	2.30, 1.96, 3.77	GLN141, LYS161, ASP162, GLN141	2.72, 1.90, 2.80, 3.12	TYR159, GLN343	2.74, 2.25	ASN21, GLY92, GLU91, GLY82	2.61, 3.00, 2.51, 2.52
10	2,2-Dimethyl-4-[(octadecyloxy) methyl]-1,3-dioxolane	GLU465	3.64	-	-	HIS160, GLU230	2.37, 3.36	ARG95	3.28
11	2-Nitro-4-nonanol	THR206, HIS386, HIS388, TYR385	2.39, 3.50, 3.72, 3.29	HIS624, ASP166	2.29, 2.90	TYR159, HIS160, HIS164	2.13, 2.14, 3.56	ARG95	3.75
12	3-(4-Methylphenyl) glutamic acid	GLN350, HIS351, LYS358	2.99, 2.84, 2.28	GLN611	2.39	ASP318, HIS160	2.72, 3.49	LEU96, GLY82, GLY17, ARG95, LU91	2.06, 2.16, 3.59, 3.51, 2.53
13	3,4-Dihydro-2H-1,6-benzoxazocin-5 (6H)-one	SER530	2.48	-	-	TYR159	2.49	GLY17	3.50
14	6-Gingerol	ARG44, CYS41	2.05, 2.42	GLN611, GLN611, GLN611, ARG401, GLU614	2.74, 2.18, 2.65, 1.94, 3.30	HIS160, ASN321	2.26, 3.57	GLY92, LEU96	2.63, 1.99
15	Dihydro-1H,5H-dipyrrolo[1,2-a1',2'-d] pyrazine-2,5,7,10 (3H,8H,10aH)-tetrone	LYS468, ARG44	3.25, 3.31	SER447	3.69	TYR159, HIS160, ASP318	2.63, 2.40, 3.38	LEU96, GLY82, GLY82	3.18, 3.65, 3.75
16	Ethyl 2-phenylethyl butylphosphonate	ALA527	3.28	HIS367	2.43	HIS160	2.34	-	-
17	Maltol	HIS207	2.77	PRO565	3.28	-	-	GLU91, GLY82, LEU96	2.12, 2.48, 2.06
18	Methyl 3-[4-(2-methoxy-2-oxoethyl) -4-methyl-2,5-dioxo-3-pyrrolidinyl] propanoate	LYS83, TYR115, SER119	2.47, 2.47, 1.94	GLN141, ARG165, ASP166, GLN168, ASP166, THR104	2.45, 2.56, 2.53, 3.02, 3.09, 3.79	TYR159, THR333	2.74, 3.74	ASN21, ASN21, GLU91, GLY92, GLU91	2.49, 2.47, 2.35, 2.46, 3.11

(*Contd...*)

Table 7: (*Continued*)

Sl. No.	Name of the compound	COX-2 (3 mdl)		5-LOX (6 ncf)		PDE4 (4 wcu)		HP5 (1 hd2)	
		Hydrogen bonds	Distance (Å)	Hydrogen bonds	Distance (Å)	Hydrogen bonds	Distance (Å)	Hydrogen bonds	Distance (Å)
19	N-Benzylformamide	HIS207, TYR385	2.74, 2.64	ILE404, ALA405, TYR383	2.10, 2.21, 2.86	THR333, ASN321	3.25, 2.77	GLY92, GLU16	2.36, 2.51
20	Phosphoglycolate	HIS207, HIS388, ASN382, ASN382	2.38, 2.81, 2.73, 2.00	TRP102, TYR81, TYR100, TYR100, ASP166, GLN168	2.14, 2.31, 2.29, 2.16, 2.12, 2.82	HIS204, THR27, HIS164, ASP201, GLU230	1.98, 2.45, 2.80, 1.99, 2.66	GLY17	3.58
21	Traumatic Acid	TYR355	1.84	ASP285	2.78	HIS160, TRP332	2.46, 3.10	LYS63, LYS63, LYS63, LYS63, LYS63, LYS63, GLU91, LYS93	2.72, 2.49, 2.65, 2.98, 2.65, 2.98, 2.13, 2.44
22	2-Aminoadipic acid	THR206, HIS207, TYR385	2.98, 2.54, 2.41	TRP102, HIS624, ASP166, TYR383, TYR81, TYR100	2.23, 2.17, 2.99, 2.70, 2.34, 2.41	TYR159, HIS160, ASP318, ASP318	2.35, 2.22, 2.33, 2.16	LYS63, LYS63, LYS63, LYS63, LYS63, LYS63, VAL70, GLY92, GLN68	2.49,2.83, 2.62, 2.60, 2.17, 2.93, 2.17, 2.93, 2.34, 2.51, 2.15
23	2-Cyclohexyl-2-hydroxyacetamide	THR206, HIS207, TYR385	3.26, 2.32, 2.83	HIS624, TYR81, TYR100, TYR100	2.39, 2.72, 1.95, 2.45	HIS160, ASP318, ASP318, ASP318, ASP318	2.41, 2.52, 2.16, 2.79, 2.17	GLY92, GLU16, GLU91	2.67, 2.18, 3.24
24	4-Undecylbenzenesulfonic acid	TRP387, HIS388	2.38, 3.61	-	-	HIS204, ASP201	2.39, 2.38	LYS63, LYS63, LYS63, LYS63, GLY92	2.15, 2.73, 2.52, 2.49, 2.71
25	9-Oxo-10(E),12(E)-octadecadienoic acid	ARG222, PHE210	3.36, 2.45	LEU448, THR545, SER447	2.50, 2.35, 3.65	GLN433, THR333	2.63, 2.52	LYS63, LYS63, LYS63, LYS63, LYS63, LYS63, VAL70	2.74, 2.53, 2.68, 3.00, 2.68, 3.00, 2.77
26	Oleamide	HIS207, THR206	2.38, 2.70	-	-	HIS160, HIS204, ASP201	2.65, 2.26, 2.19	LYS63, LYS63, LYS63, VAL70, VAL70, LYS93	2.18, 2.55, 2.55, 2.63, 2.86, 2.52
27	Pyrogallol	-	-	THR545, ARG370	2.99, 4.05	-	-	LYS63, LYS63, LYS63, LYS63, LYS63, GLY92, VAL70, GLY92	2.45, 2.79, 2.37, 2.02, 2.02, 2.20, 2.18, 2.86

ALA: Alanine, ARG: Arginine, ASN: Asparagine, ASP: Aspartic acid, GLN: Glutamine, GLU: Glutamic acid, GLY: Glycine, HIS: Histidine, LEU: Leucine, LYS: Lysine, MET: Methionine, PHE: Phenylalanine, PRO: Proline, SER: Serine, THR: Threonine, TRP: Tryptophan, TYR: Tyrosine, VAL: Valine

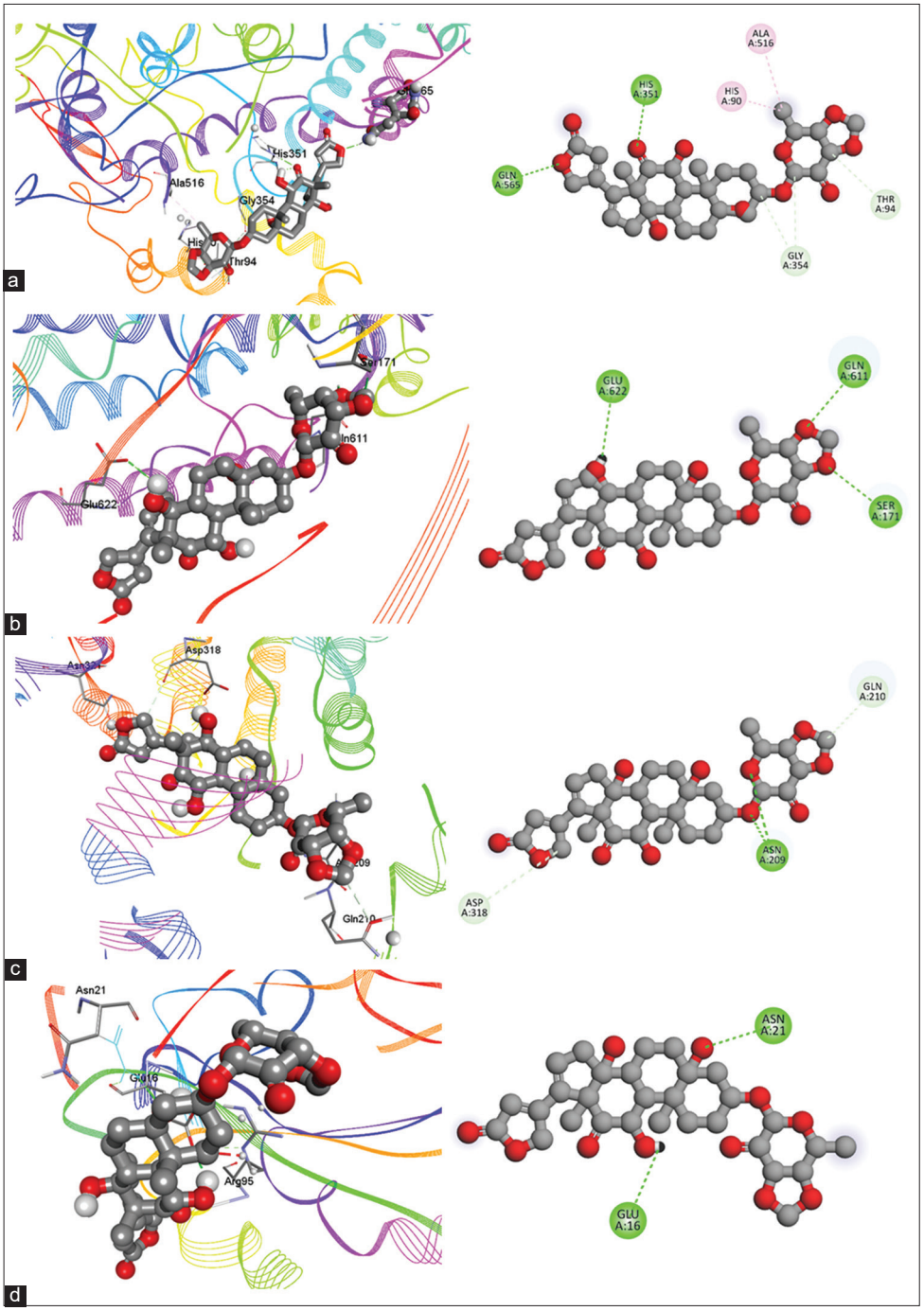

Fig. 9: Molecular interactions and binding affinities of Anodendroside E 2 with (a) cyclooxygenase-2, (b) 5-lipoxygenase, (c) Phosphodiesterase 4, and (d) Human peroxiredoxin 5

The hydrogen bond interactions between Triamcinolone Acetonide and COX-2 occur through specific atoms of both the ligand and the enzyme's amino acid residues. In the case of HIS351, the nitrogen atom in the imidazole ring interacts with a hydrogen donor from the ligand, forming a bond at 2.67 Å. For GLN565, the carbonyl oxygen of Triamcinolone Acetonide interacts with the amide nitrogen of the residue, with a bond length of 2.48 Å. GLN350 forms a hydrogen bond at 2.22 Å, where the oxygen atom of the ligand's hydroxyl group interacts with the amide nitrogen of the residue. GLY354 is involved through its backbone amide group interacting with an oxygen atom from the ligand, at a distance of 3.41 Å. Finally, SER579 forms a hydrogen bond at 3.50 Å, where the hydroxyl oxygen of the SER side chain interacts with the carbonyl group of Triamcinolone Acetonide. In the case of Arginine (ARG)401, the guanidinium group of ARG interacts with an oxygen atom from the carbonyl group of Triamcinolone Acetonide, forming a hydrogen bond at 2.82 Å. For ASN613, the side chain amide group interacts with a hydroxyl group of the ligand at a distance of 2.07 Å. These atomic interactions stabilize the binding of Triamcinolone Acetonide in the active site of 5-LOX, enhancing its inhibitory effect on the enzyme. The hydroxyl groups and carbonyl oxygen atoms of Triamcinolone Acetonide are key interaction sites with the nitrogen atoms of HIS residues (HIS160, HIS204), amide groups of ASN209, hydroxyl groups of THR271, and the carboxyl group of GLU230. The molecular interactions between Triamcinolone Acetonide and HP-5 are primarily stabilized by a strong hydrogen bond with GLU91 (2.43 Å) and a weaker hydrogen

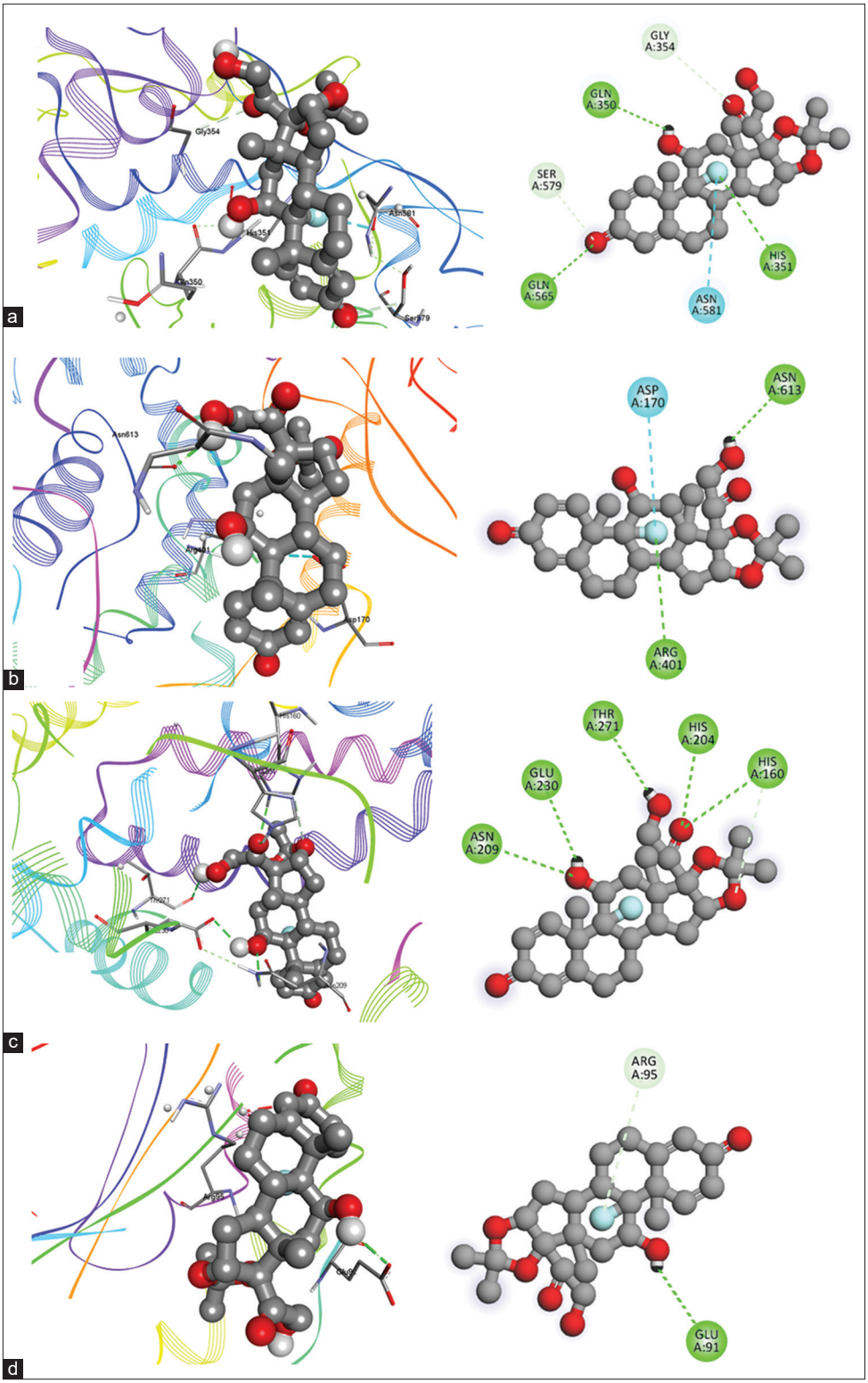

Fig. 10: Molecular interactions and binding affinities of Triamcinolone Acetonide with (a) cyclooxygenase-2, (b) 5-lipoxygenase, (c) Phosphodiesterase 4, and (d) Human peroxiredoxin 5

bond with ARG95 (3.63 Å). The 3D and 2D interactions are depicted in Fig. 10.

The interaction between Fluprednisolone and COX-2, HIS356, forms two hydrogen bonds with Fluprednisolone at distances of 3.56 Å and 3.60 Å, indicating relatively weaker interactions. GLN565 forms a strong hydrogen bond at a distance of 2.38 Å, likely involving the oxygen atom from the carbonyl or hydroxyl group of Fluprednisolone interacting with the amide group of GLN565. ASP347 forms a hydrogen bond at a distance of 3.56 Å, involving its carboxyl group interacting with an oxygen atom from Fluprednisolone. The interaction between Fluprednisolone and 5-LOX indicates a moderately strong interaction, likely involving the carboxyl group of ASP170 and a hydroxyl or carbonyl group on Fluprednisolone. In the interaction between

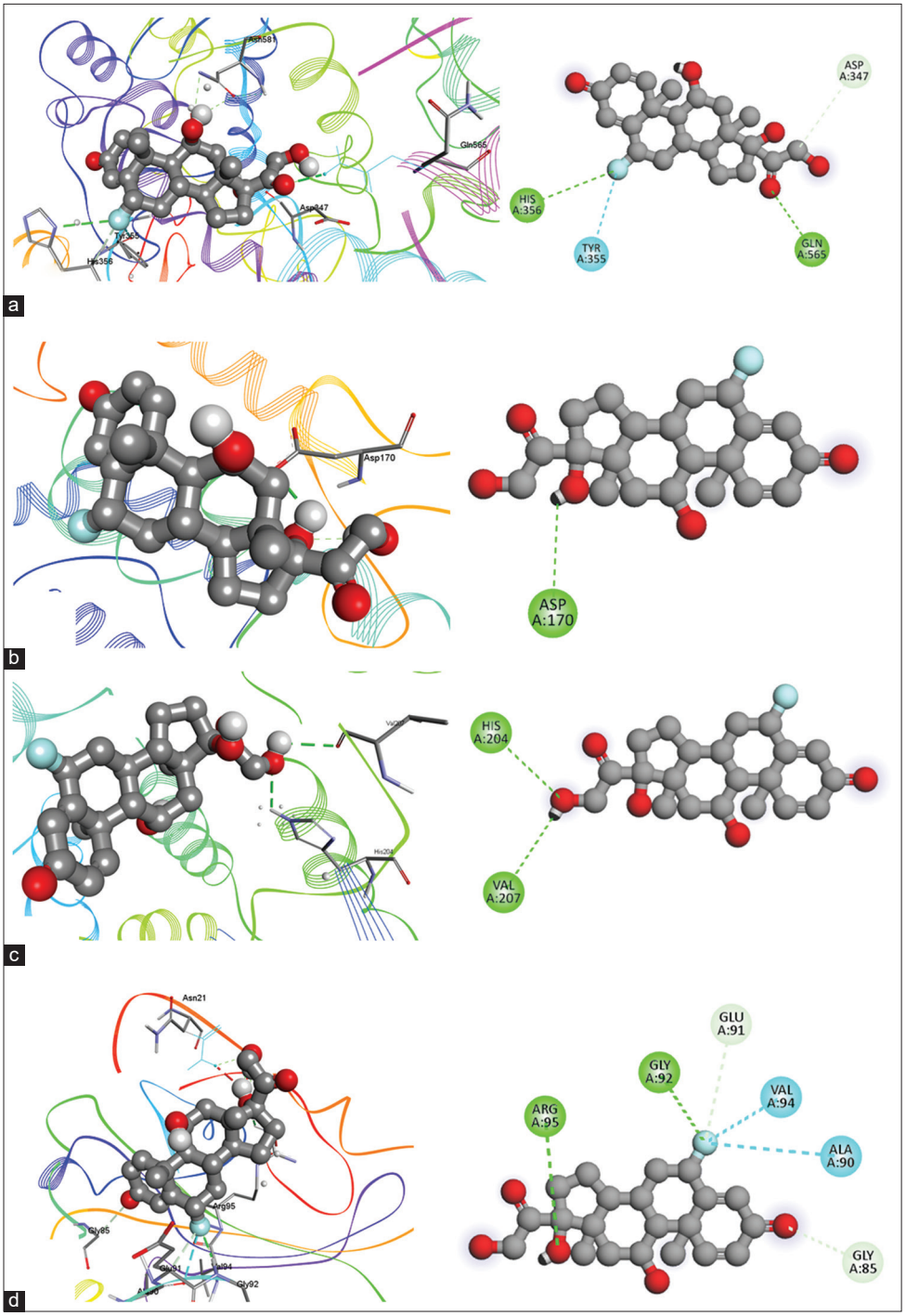

Fig. 11: Molecular interactions and binding affinities of Fluprednisolone with (a) cyclooxygenase-2, (b) 5-lipoxygenase, (c) Phosphodiesterase 4, and (d) Human peroxiredoxin 5

Fluprednisolone and PDE4, the interactions likely involve a hydroxyl group on Fluprednisolone engaging with the side chains of HIS204 and valine (VAL)207, contributing to the stabilization of the ligand within the PDE4 binding pocket. The interaction between Fluprednisolone and HP-5 involves key hydrogen bond interactions with several residues such as GLY92, ARG95, GLY85, and GLU91. The 3D and 2D interactions are depicted in Fig. 11.

The interaction between Maltol and COX-2 is mediated primarily through a hydrogen bond with the HIS207 residue. The imidazole nitrogen atom of HIS207 forms a hydrogen bond with the carbonyl oxygen atom of Maltol at a distance of 2.77 Å. The hydrogen bond forms between Maltol and the residue proline 565, and the distance of this interaction is measured at 3.28 Å, indicating a weaker hydrogen bond compared to shorter interactions. The oxygen atom of Maltol interacts with GLU91 at a bond distance of 2.12 Å, which signifies a strong interaction. In addition, Maltol's hydrogen atom is involved in hydrogen bonding with GLY82 at a distance of 2.48 Å. Another significant interaction is observed with leucine (LEU)96, where the bond distance is 2.06 Å, indicating a robust molecular interaction. These interactions stabilize Maltol within the binding pocket of HP-5. The 3D and 2D interactions are depicted in Fig. 12.

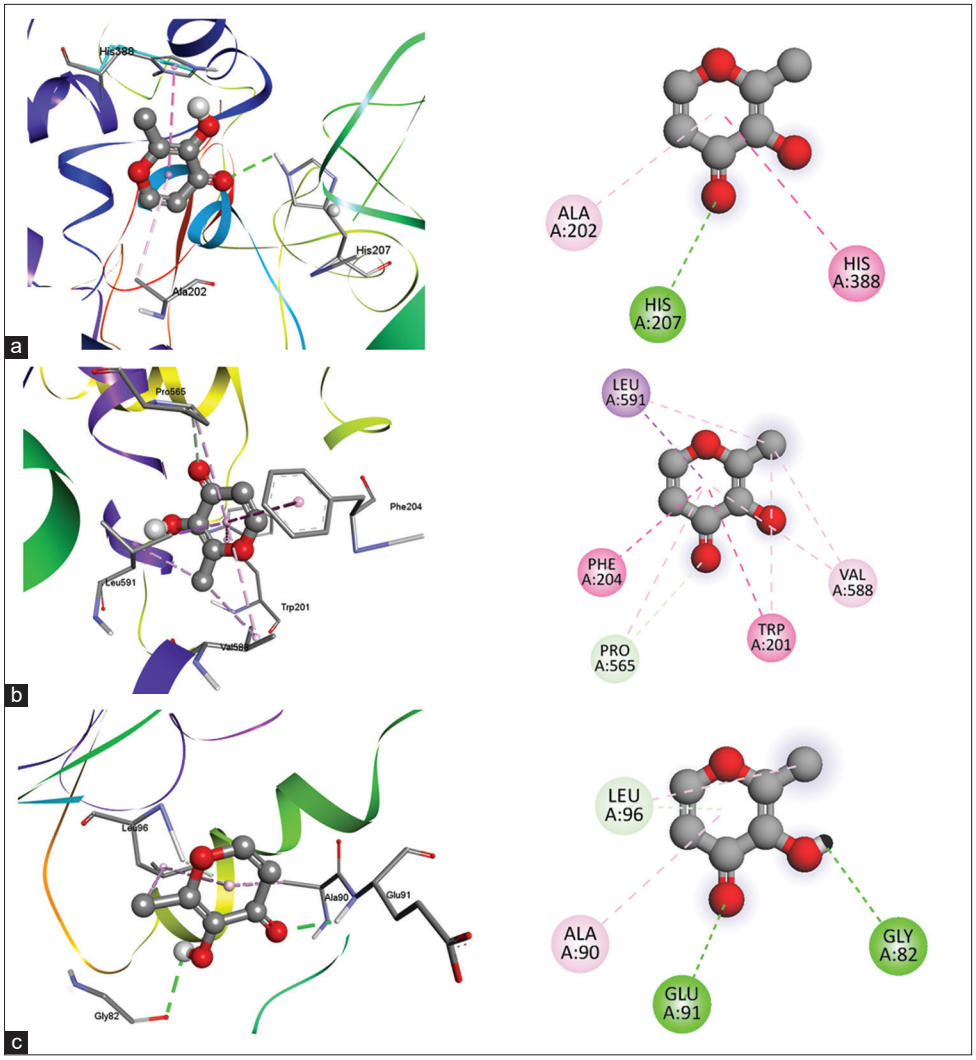

Fig. 12: Molecular interactions and binding affinities of Maltol with (a) cyclooxygenase-2, (b) 5-lipoxygenase, and (c) Human peroxiredoxin 5

The hydroxyl group of 6-Gingerol forms a strong hydrogen bond with the side chain of ARG44 at a distance of 2.05 Å. This interaction is particularly significant due to the positively charged guanidinium group of ARG, which can form stable electrostatic interactions with the oxygen atom of the hydroxyl group. Such an interaction likely plays a key role in stabilizing the binding of 6-Gingerol within the COX-2 active site. In addition, the sulfur-containing side chain of CYS41 forms another hydrogen bond with 6-Gingerol at a distance of 2.42 Å, contributing to the molecule's stabilization within the enzyme. The molecular interaction of 6-Gingerol with the 5-LOX enzyme involves several crucial hydrogen bonding interactions. The side chain of GLN611 is engaged in multiple hydrogen bonds at distances of 2.74 Å, 2.18 Å, and 2.65 Å. The presence of hydrogen bonding between the hydroxyl group of ARG401 at 1.94 Å and the hydroxyl group of 6-Gingerol suggests a strong electrostatic interaction. The distance of 3.30 Å between GLU614 and 6-Gingerol further complements the network of interactions. These interactions likely involve functional groups on the 6-Gingerol molecule such as hydroxyl (OH) and carbonyl (C=O) groups. 6-Gingerol forms a hydrogen bond with HIS160 at a distance of 2.26 Å, indicating a strong interaction. There is a weaker interaction with ASN321 at a distance of 3.57 Å. In the context of 6-Gingerol interacting with HP5, the hydrogen bond is formed between the oxygen atom of the carbonyl group in GLY92 and a hydrogen atom of 6-Gingerol. This interaction occurs at a bond distance of 2.63 Å. In addition, LEU96 participates in another hydrogen bond with 6-Gingerol, where a close interaction is observed at a bond distance of 1.99 Å. The 3D and 2D interactions are represented in Fig. 13.

The oxygen atom of 2-Aminoadipic acid interacts with THR206 at a bond distance of 2.98 Å, another interaction occurs between the oxygen atom and the amino acid HIS207, with a bond distance of 2.54 Å, forming a stable hydrogen bond. The hydrogen bond is formed between the nitrogen atom of 2-Aminoadipic acid and tyrosine (TYR)385, with a bond distance of 2.41 Å. These functional groups facilitate the formation of stable hydrogen bonds that anchor 2-Aminoadipic acid in the active site, influencing the binding affinity and specificity of the interaction with COX-2. The interactions between the polar functional groups of 2-Aminoadipic acid (such as its amino group and carboxyl group) and the amino acid residues of 5-LOX provide a stable network of hydrogen bonds. The amino group (-NH2) of 2-Aminoadipic acid plays a crucial role in forming hydrogen bonds with polar side chains, such as the imidazole ring of HIS624 and the hydroxyl groups of tyrosine residues (TYR81, TYR100, and TYR383). This interaction helps to anchor the ligand in the active site. The carboxyl group (-COOH) of 2-Aminoadipic acid can participate in ionic interactions with negatively charged residues, particularly with ASP166. In the molecular interaction between 2-Aminoadipic acid and PDE4, the carboxyl group of 2-Aminoadipic acid forms hydrogen bonds with key residues such as TYR159 (at 2.35 Å) and HIS160 (at 2.22 Å). In addition, the same functional group interacts with ASP318, forming two hydrogen bonds at distances of 2.33 Å and 2.16 Å, respectively. In the molecular

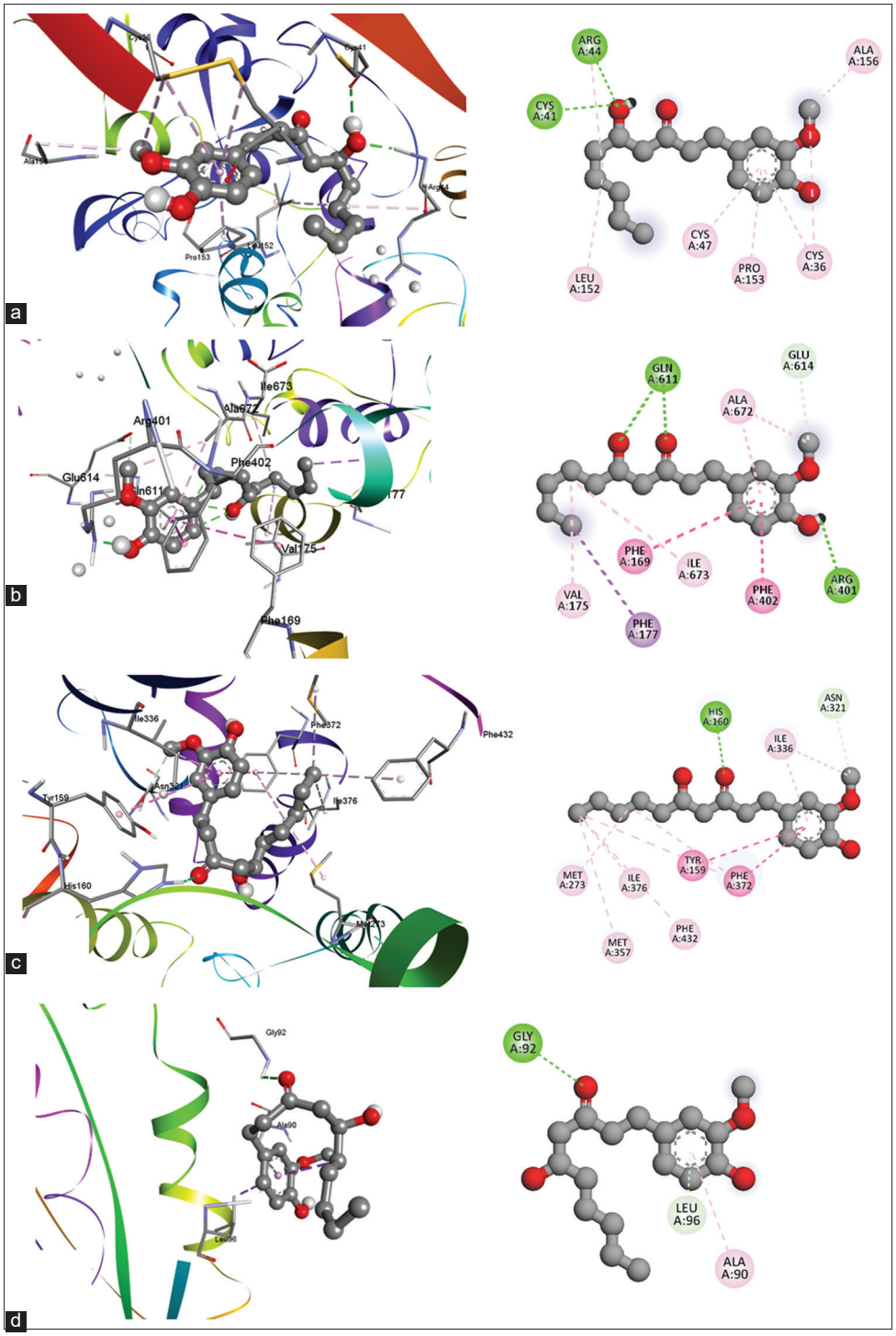

Fig. 13: Molecular interactions and binding affinities of 6-Gingerol with (a) cyclooxygenase-2, (b) 5-lipoxygenase, (c) Phosphodiesterase 4, and (d) Human peroxiredoxin 5

interaction between 2-Aminoadipic acid and HP5, the carboxyl group forms a hydrogen bond with GLY92, with a bond distance of 2.19 Å. In addition, the carbonyl group interacts with VAL70 and lysine (LYS)63, forming hydrogen bonds at distances of 2.65 Å and 3.28 Å, respectively. The interaction with GLN68 and another hydrogen bond with LYS63. The 3D and 2D interactions are represented in Fig. 14.

The results demonstrated that the compounds identified from GC-MS and LC-MS analysis, including Triamcinolone Acetonide, Fluprednisolone, Maltol, 6-Gingerol, and 2-Aminoadipic acid, previously reported for their anti-inflammatory activity, showed significant binding affinity through both hydrogen and hydrophobic interactions with key targets such as COX-2, 5-LOX, PDE4, and HP5. These strong interactions with crucial inflammatory enzymes highlight their potential as effective anti-inflammatory agents. Further studies targeting other enzymes will provide more insights into the anti-inflammatory properties of *U. distachya*.

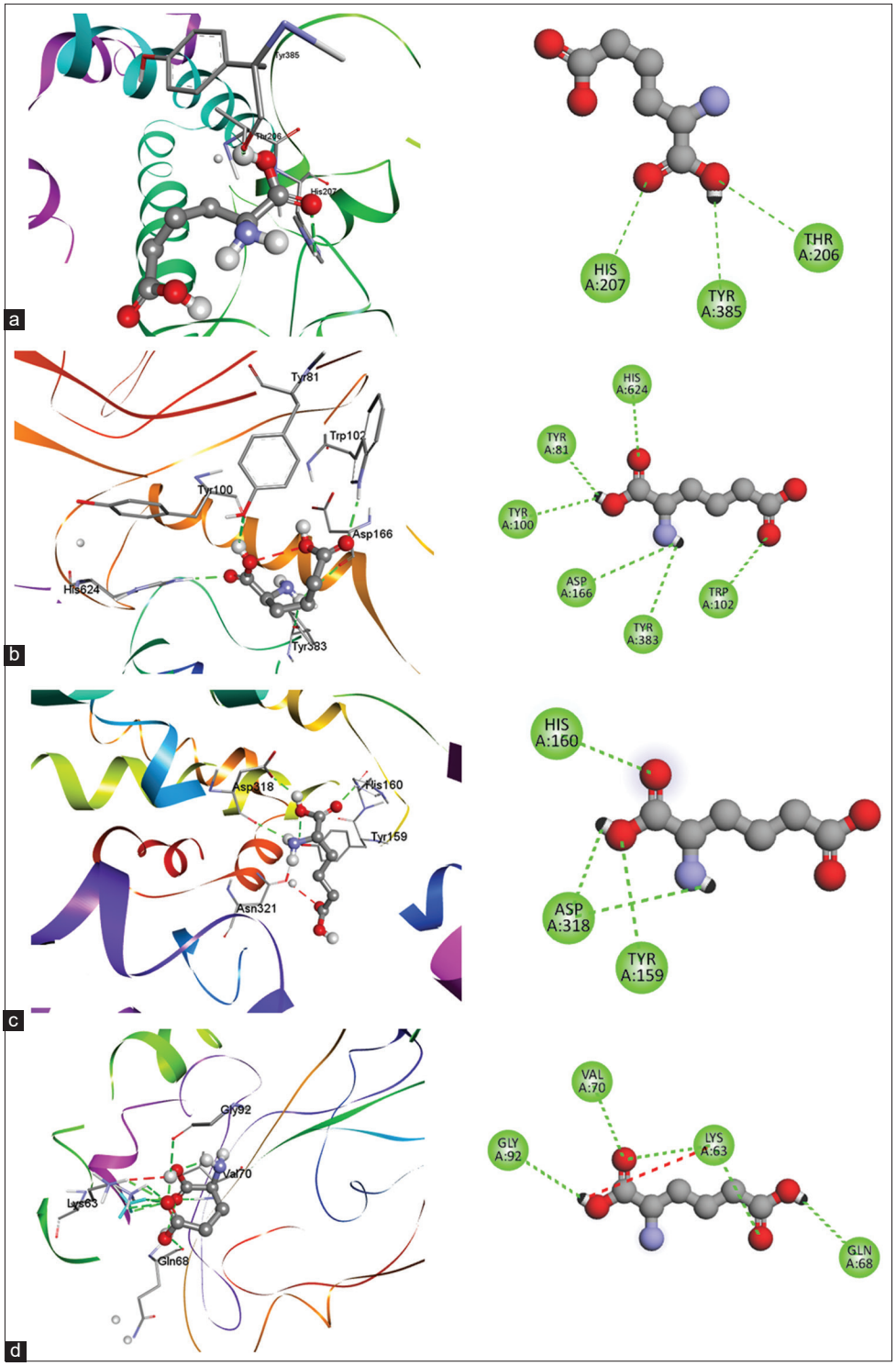

Fig. 14: Molecular interactions and binding affinities of 2-Aminoadipic acid with (a) cyclooxygenase-2, (b) 5-lipoxygenase, (c) Phosphodiesterase 4, and (d) Human peroxiredoxin 5

CONCLUSION

The present study highlights the anti-inflammatory potential of phytoconstituents identified from CUD and EAUD extracts through GC-MS and LC-MS analyses. Both extracts, particularly CUD at a dose of 400 mg/kg body weight, exhibited significant reductions in carrageenan-induced paw edema in rats, with CUD showing more potent activity compared to EAUD. *In silico* molecular docking studies further supported these findings, revealing strong binding affinities of compounds such as Holothurin acetate, 7, 8-Epoxylanostan-11-ol, 3-acetoxy-, 7,8,12-Tri-O-acetyl-3-desoxy-ingol-3-one, Anodendroside E 2, Triamcinolone Acetonide, Fluprednisolone, Maltol, 6-Gingerol, and 2-Aminoadipic acid with key inflammatory targets including COX-2, 5-LOX, PDE4, and HP5. The binding affinities of these compounds suggest promising anti-inflammatory properties. Further molecular simulations and *in vivo* models are necessary to validate these findings and assess the true therapeutic potential of these compounds. This combined approach could lead to the development of new anti-

inflammatory agents based on the bioactive compounds identified in this study.

CONSENT FOR PUBLICATION

Not applicable.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Animal Ethical Committee (1376/PO/Re/S/10/CPCSEA) approved the study protocol and confirmed the guidelines for animal care and use in scientific research. The project proposal numbers IAEC/01/2024 for the acute toxicity study and IAEC/03/2024 for carrageenan-induced paw edema in rats.

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CONFLICTS OF INTEREST

The author declares that they have no conflicts of interest.

FUNDING

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