Future Journal of Pharmaceutical Sciences

RESEARCH Open Access

Scrutinizing pharmacological efficiency for *Acacia auriculiformis* by experimental and computational approach

Shafinaz Nur†, Md. Mohotasin Hossain†, Nadia Islam, Abu Montakim Tareq, Nujhat Binte Hanif, Riniara Khatun and Mohammed Abu Sayeed*

Abstract

Background: The study sought to investigate the biological efficacy of methanol leave extract of *Acacia auriculiformis* (MEAA) via in vitro, in vivo, in silico approaches. The in vitro cytotoxicity was evaluated through brine shrimp lethality assay, and anti-inflammatory activity was determined by membrane stabilisation and protein denaturation methods (BSA and egg albumin). The in vivo antipyretic activity was examined via Brewer's yeast induced pyrexia model.

Results: *A. auriculiformis* extract unveiled moderate cytotoxicity with significant anti-inflammatory efficacy ($p < 0.001$) compared to standard drug. This extract also exhibited dose-dependent time of paralysis and death for the worm ($p < 0.001$) in the anthelmintic test which was directly proportional to employed concentrations. A notable percentage of clot lysis effect (36.42 ± 1.95%, $p < 0.001$) was also observed for MEAA in human blood compared to control. However, this extract significantly ($p < 0.05$) reduced fever in a dose-dependent manner during the antipyretic experiment. Besides, in computer-aided investigations, two compounds (2,4-ditert-butylphenol and 3-hydroxy-β-damascone) revealed the best binding interaction with six proteins for cytotoxicity, inflammation, helminthic, thrombolytic and pyretic effect. Moreover, these two compounds satisfy Lipinski's 'Rule of Five' and revealed drug-likeness profiles in the toxicological study.

Conclusions: These findings disclosed that methanol leaves extract of *A. auriculiformis* might be a potent source for anti-inflammatory, anti-helminthic, thrombolytic and antipyretic agents.

Keywords: *Acacia auriculiformis*, Anti-inflammatory, Anthelmintic, Thrombolytic, Antipyretic

Background

Inflammation is a biological defensive response initiated by several stimuli, including irritants, chemicals, heat, infection or any immunological reactions [1]. In general condition, inflammation shows hyperthermia and pain in the living body and aims to repair damaged tissue by inactivating deleterious stimulus or pathogen. Hence, uncontrolled acute inflammation for a prolonged period can cause drastic pain and fever in the body. These can be pathophysiology of severe diseases like cancer, rheumatoid joint pain, asthma, diabetes, multiple sclerosis, autoimmune diseases, ischemic heart disease, arthritis, atherosclerosis and Alzheimer disease [2]. However, several inflammatory mediators like prostaglandins, bioactive lipid derivative are responsible for mediation of clotting, pain vasodilation, and pyrexia [3].

Pyrexia is a complex immune biochemical reaction that initiates by producing various endogenous pyrogens as a response to inflammatory or infectious stimulation [4]. The presence of pathogens or tissue damage in the body activates the immune system which produces and releases pyrogenic cytokines. In the hypothalamus, these

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synthesise cytokines react with prostaglandin receptors and alter the thermoregulation system. This alteration results in increased body temperature beyond the set point of 37.2 °C under the arm or axillar, 37.5–38.3 °C in the anus or rectum, 37.7 °C in the mouth and affects normal body functions [5]. Several antipyretic and anti-inflammatory agents like paracetamol, ibuprofen and NSAIDs are widely prescribed to reduce excessive body temperature [6]. These agents mainly exert their activity by inhibiting selective and non-selective prostaglandin and cyclooxygenase enzymes. Hence, long time inhibition of these enzymes can lead to serious consequences, including iatrogenic reactions, neutropenia, GIT disorder, kidney dysfunction, liver failure and other bleeding disorders [7]. A recent alternative strategy is the inhibition of microsomal mPGES-1 (prostaglandin E synthase-1), but till now, only two inhibitors were found [8]. For these reasons, a new therapeutic approach having multifaceted pharmacological targets and less side effect has been becoming an ultimate focus for researchers all around the world.

Since the hunter-gatherer age, humans have been using natural herbs to treat various diseases [9]. Natural products have always been a vital health concern due to their phytochemicals and nutritional values as they are a decent source of antioxidants, phenolics, vitamins, fibres, minerals, bioactive metabolites [10]. In many instances of modern synthetic drug development, phytoconstituents derived from natural plants screening program assists meticulously [11]. As plant metabolites contain elevated amounts of bio-chemical compounds with effective complementary pharmacological activities, their practice in alleviating chronic diseases is becoming a great area of interest [12]. Hence, appropriate strategic research on medicinal plants to discover novel bioactive agents is inevitable. Regarding this indigenous point of view, we aimed to investigate an ethnomedicinal plant *Acacia auriculiformis,* in our current study.

Acacia auriculiformis is locally known as Akashmoni in Bangladesh and babul in India. *A. auriculiformis* belongs to Plantae kingdom, Viridiplantae subkingdom, Streptophyta infrakingdom, spermatophyte phylum, *Acacia mill* genus and fabaceae/leguminosae family. Traditionally, this plant is used to treat rheumatism, aches, anthelmintic, pain, sore eyes, candidiasis and conjunctivitis [13]. Australian aborigines use root decoction of this plant in the treatment of rheumatism [14]. Seeds of this plant are traditionally used in the treatment of skin ailment, rashes, itching and allergy. In Nigeria, the Ibibio community uses this plant against malaria. Several phytochemicals, including flavonoids, carbohydrates, tannins, saponins and anthocyanidins, have been reported from *A. auriculiformis* [13]. Isolated compounds, namely acaciaside A and B, exhibited moderate anti-microbial and anti-fungal activity via in vitro assay [15]. However, ethanolic leave extract has shown dose-dependent anti-plasmodial, anti-microbial, larvicidal activity along with memory improvement and acetylcholinesterase (AChE) enzyme inhibition in rats [16, 17]. Besides, Sathya et al. 2013 reported that petroleum ether extracts of empty pod and bark from this plant are potent candidates for diabetes and liver injury [18].

The plant *A. auriculiformis* possess several suitable medicinal properties, but until now, no study has been done reporting anti-inflammatory, anthelmintic, thrombolytic and antipyretic activities of this plant. Therefore, our present study aims to systematically explore these activities for methanol leaves extract of *A. auriculiformis* (MEAA). Most importantly, the current investigation aims to find out the therapeutic intervention of MEAA in mitigating inflammation by fever is the ultimate aim of our experiment. Additionally, a computational approach (molecular docking, ADME/T) was performed to predict possible insight targets of bioactive constituents of MEAA for a conceivable perspective.

Methods

Crude extract preparation

A. auriculiformis leaves were collected and identified from Rangamati region during September 19 and authenticated by a taxonomist under the accession number Anwar-0870. The leaves were washed and dried in a shaded place under 55–60 °C for 6 days. Coarse powder (490 g) was obtained from dried leaves by using an electric grinder (Sahara mixer grinder, Canbara industries PVT. Ltd., Mumbai-400072). The coarse powder was soaked in an appropriate amount (1000 mL) of methanol (90%) for 7 days with occasional shaking and stirring at room temperature. The filtration was done by Whatman No. 1 filter paper in a laboratory condition 27 ± 2 °C. The finale filtrate was allowed to dry and evaporate in a water bath at 45–50 °C. Four gram sticky, greenish, semisolid extract was found and preserved in 4 °C until further use as a methanol extract of *A. auriculiformis* (MEAA) [7].

Chemicals

Streptokinase vial (1,500,000 IU), loperamide and vincristine sulfate (1 mg/vial) WERE obtained from Beacon Pharmaceuticals Ltd. Bangladesh. Methanol (Merck, Germany), Tween 80 and DMSO were purchased from BDH Chemicals. Bovine serum albumin and diclofenac sodium were purchased from Square Pharmaceuticals Ltd. Normal saline solution (0.9% NaCl) was obtained from Orion Infusion Ltd. Other chemicals were procured from local sources. All the chemicals were in analytical grade.

Experimental animal

Swiss albino male mice (weight: 25–30 g) were procured from an appropriate source. All mice were housed in a laboratory for 14 days to familiarise with the standard environment. Standard guidelines such as room temperate 25 ± 2 °C, relative humidity 55–60%, 12 h light/dark cycle, whereas the food pellets and water supply were maintained strictly until the experiment period. Using diethyl ether anaesthesia, the mice were euthanised after the experiment. All the mice have been slaughtered using diethyl ether anaesthesia at the end of the observation period. The ARRIVE guidelines are in compliance with for the mice model study.

In vitro study

Cytotoxicity activity by brine shrimp lethality bioassay

In vitro brine shrimp lethality bioassay for MEAA was performed by the method delineated by Meyer et al. 1982 [19]. Artificial seawater was obtained by dissolving NaCl (38 g) in distilled water (1000 mL). The pH at 8.0 was attuned by adding NaOH. MEAA (5 mg/mL) concentrations (31.25, 62.5, 125, 250 and 500 μg/mL) were obtained via serial dilution. Similarly, as a standard control, serially diluted vincristine sulfate (3.13, 6.25, 12.5, 25 50 and 100 μg/mL) was used. After obtaining the concentrations, ten alive brine shrimp were placed in each tube at 25 ± 1 °C. The number of alive nauplii was counted and recorded after 24 h by using the magnifying glass. The following equation used to determine the % of mortality,

$$\text{Mortality}\% = \frac{\text{Nt} - \text{Na}}{\text{Nt}} \times 100$$

Here, Nt = number of nauplii taken, Na = number of nauplii alive

Anti-inflammatory test on protein denaturation inhibition by egg albumin

The anti-inflammatory activity of MEAA was studied by the egg albumin inhibiting protein denaturation method used by Guha et al. 2020 with slight modification [20]. The reaction solution (5 mL) contains egg albumin (0.2 mL), phosphate-buffered saline at pH 6.4 (2.8 mL) and MEAA extract (2 mL) varying concentration of 62.5, 125, 250 and 500 μg/mL. The exact volume of distilled water was used as a control group. In a BOD incubator (Lab-line technology, Japan), all the solutions were incubated for 15 min at 37 ± 2 °C and heated for 5 min at 70 °C. After cooling, the absorbance was measured at 660 nm (SHIMADZU, UV 1800) where water (vehicle) was used as a control and diclofenac sodium concentrations (31.25, 62.5, 125, 250 and 500 μg/mL) used as a reference solution. The test was carried out triplicate times, and the following equation was used to determine the percentage of protein denaturation inhibition,

$$\text{Inhibition}\ (\%) = \frac{\text{Abs control} - \text{Abs test}}{\text{Abs control}} \times 100$$

Abs control = absorbance of the control, Abs test = Absorbance of test

Anti-inflammatory test on protein denaturation inhibition by BSA

The anti-inflammatory activity of MEAA was studied by BSA inhibiting protein denaturation method formerly used by Tareq et al. 2020 with minor modifications [9]. The test mixture (0.5 mL) composed with 0.45 mL aqueous albumin (5% w/v) and 0.05 mL of MEAA extract while control mixture (0.5 mL) contain 0.45 mL aqueous albumin (5% w/v) and 0.05 mL of distilled water. The product mixture was composed of 0.05 mL of the test mixture and 0.45 mL of distilled water. The standard mixture (0.5 mL) comprises 0.05 mL of diclofenac sodium instead of distilled water. Different concentration for both test and standard mixture was obtained (125, 250, 500 and 1000 μg/mL) and pH was attuned to 6.3 by adding 1 N HCl in all concentrations. After that, all mixture was incubated for 20 min at 37 °C and then kept for 30 min at 57 °C. Finally, after cooling the mixture, phosphate buffer (2.5 mL) was added to each mixture and absorbance was recorded at 416 nm by using a UV-visible spectrophotometer. The test was carried out triplicate times and the following equation determined the percentage of protein denaturation inhibition,

$$\text{Inhibition}\ (\%) = 100 - \left(\frac{A - B}{C}\right) \times 100)$$

A = Absorbance of test mixture; B = Absorbance of product mixture; C = absorbance of control mixture.

Anti-inflammatory test on membrane stabilisation by hypotonic induced haemolysis

In vitro membrane stabilisation activity for MEAA was assessed by the described method Sabiu et al. 2016 [21]. The text mixture was serially diluted at 31.25, 62.5, 125, 250, 500 and 1000 μg/mL concentrations. Each concentration contains MEAA (0.5 mL), phosphate buffer (1 mL), hyposaline (2 mL) and human red blood cell (HRBC) suspension (0.5 mL). The standard mixture (diclofenac sodium) contains the same chemicals except for the extract, whereas the control mixture contains all chemicals except the standard drug and extract. All the mixtures were incubated for 30 min at 37 °C. After cooling, centrifugation was for 20 min at 3000 rpm. Therefore, absorbance was taken for obtained supernatant solutions by using UV-visible spectrophotometer at 540

nm. The inhibitory haemolysis effect of MEAA was determined via the following equation

$$\text{Membrane stability} = \left(\frac{A - B}{A}\right) \times 100$$

Here, A = absorbance of control mixture, B = absorbance of test mixture.

In vitro anthelmintic activity on the worm *Tubifex tubifex*

The anthelmintic activity of MEAA was evaluated by the method previously used by Adnan et al. 2018, with contemptible modifications [22]. The worms (*Tubifex tubifex*) were collected from aquarium shop Chittagong with 2–2.5 cm average length. The test groups contained concentrations of MEAA (5, 8 and 10 mg/mL), whereas the control group consists of distilled water and the standard group contains levamisole (1 mg/mL). Five groups were divided into five petri dish each contains randomly selected ten worms. For each aimed groups, 3 mL of the mixture was added. After that, 'time of paralysis' and 'time of death' of worms were observed and recorded carefully. When the worms stopped movement but keep vigorous shaking was considered as 'time of paralysis', and the period when the worms completely stopped movement was regarded as 'time of death'. The experiment was conducted triplet time.

In vitro thrombolytic activity via clot lysis

In vitro clot lysis activity was assessed by the described method of Prasad et al. 2006 [23]. As a test sample, MEAA crude extract (100 mg) was mixed with 10 mL distilled water and kept overnight. Streptokinase (1,500, 000 IU) was used as a stock solution. A total of 5 mL venous blood was drawn from 10 healthy and young volunteers (no history of any substance/drug used in the past 7 days). Blood retrieved from each volunteer was dispersed in pre-weighed Eppendorf tubes. Each Eppendorf containing 0.5 mL blood was incubated for 45 min at 37 °C to form a clot. After incubation, layers of clot and serum were formed in each Eppendorf tubes. Serum was carefully aspirated out from each tube without disturbing the clot and the weight of tubes containing clots was measured. Therefore, 100 μL of MEAA (10 mg/mL) solution was added to each tube, whereas in the standard control tube streptokinase (SK) (100 μL), and in the control group distilled water (100 μL) was used. Then all the tubes were incubated for 90 min at 37 °C. The released fluid from each tube again removed after incubation without distressing the clot. Hereafter, all the tubes weighted again to observe clot disruption. The equation described by Banu et al. 2020 [24] was followed to determine the percentage of clot lysis.

In vivo study

Antipyretic test using Brewer's yeast induced pyrexia mice model

The antipyretic activity of MEAA was ascertained by the method previously used by Ahmed et al. 2019 [25]. Sixteen Swiss albino rodents were taken randomly for our current experiment and identified numerically. Then, by using a digital thermometer, the initial body temperature for all of the rodents was recorded. In the initial period, the temperature 37.25 °C is considered as usual and above 37.50 °C was considered as fever. Twenty percent of an aqueous suspension of Brewer's yeast (10 mL/kg, s.c.) was injected into all rodents to induce pyrexia. After pyrexia induction, all rodents were fasted overnight with only access to drink water. After 18 h, the temperature was again observed and recorded for each rodent via a digital thermometer. An increment of the temperature of more than 0.5 °C confirms the induction of pyrexia [26]. Therefore, rodents that did not show temperature increment were excluded from the current experiment. Hereafter, twelve male pyrexia-induced Swiss albino mice were divided into four groups, each containing three rodents. Group I was considered negative control and received 10 mL/kg saline, whereas group II was considered a control group and received paracetamol (150 mg/kg). The remaining two groups (III and IV) were considered test groups where group III received an MEAA dose of 200 mg/kg and group IV received an MEAA dose of 400 mg/kg. After drug administration, rectal temperature was again recorded and observed for 1 h, 2 h, 3 h and 4 h periodically. Using diethyl ether anaesthesia, the mice were euthanised after the experiment. All the mice have been slaughtered using diethyl ether anaesthesia at the end of the observation period. The percentage of pyrexia reduction was determined by the following equation,

$$\%\text{reduction} = \frac{PiT - Cn}{PiT - NbT} \times 100$$

Here, PiT = Pyrexia induced body temperature, Cn = temperature after 1 h, 2 h, 3 h, 4 h, NbT = normal body temperature.

Molecular docking

Formerly isolated compounds from leaves extract of *A. auriculiformis* via GC-MS analysis were exploited for molecular docking by following previously elucidated protocol Rahman et al. 2020 [12, 27]. Three-dimensional structures of the following proteins were retrieved from Protein Data Bank [28]: cyclooxygenase (COX)-1 (PDB ID: 2OYE) [29], cyclooxygenase (COX)-2 (PDB ID: 6COX) [30], microsomal prostaglandin E synthase (mPGES)-1 (PDB ID: 3DWW) [31], human estrogen

receptor (PDB ID: 1ERR) [32], tubulin-colchicine (PDB ID: 1SA0) [33], tissue plasminogen activator (PDB ID: 1A5H) [34]. Molecular docking study was analysed by Schrödinger Maestro (v11.1).

ADME/T and toxicological properties analysis

The drug-like attributes (ADME/T) of the compounds were analysed based on Lipinski's rules of five by using the tool QikProp (Schrödinger v11.1) [35]. Toxicological properties were evaluated via admetSAR (Online tool) [36].

Statistical analysis

Data were uttered as mean ± SEM (standard error of the mean). The one-way statistical analysis of variance (ANOVA) was done by following post hoc Dunnett's test for multiple comparisons. In the outcomes, $p < 0.05$, $p < 0.01$ and $p < 0.001$ are considered as statistically significant for all cases. The analysis was carried out by using either the statistical software package for social science (SPSS, version 25.0, IBM Corporation), Prism version 8.4.2 (GraphPad Software Inc., La Jolla, CA, USA), or Microsoft Excel '13.

Results

Effect of MEAA on brine shrimp lethality bioassay

To determine the cytotoxicity of MEAA, brine shrimp lethality bioassay was performed in the current study. Results demonstrates that the MEAA exhibited moderate cytotoxicity (LC_{50} = 426.38 µg/mL) against positive control vincristine sulfate (LC_{50} = 39.25 µg/mL). The lethal median dose (LC_{50}) was calculated from the regression analysis curve and the best fit line is visualised in Fig. 1.

Effect of MEAA on protein denaturation inhibition by egg albumin

The results demonstrates that for 62.5, 125, 250 and 500 µg/mL concentrations, MEAA showed 47.05%, 42.89%, 41.42%, 40.68% inhibition of egg albumin whereas, diclofenac sodium showed 83.5%, 77.96%, 67.77% and 65.4% inhibition. Statistically, both MEAA and diclofenac sodium concentrations revealed significant ($p < 0.001$) egg albumin inhibition. The results are demonstrated in Fig. 2.

Effect of MEAA on protein denaturation inhibition by BSA

In albumin bovine serum denaturation method, different concentrations of MEAA was found significant ($p < 0.001$) compared to a control mixture. The results reveals that at different concentrations (125, 250, 500 and 1000 µg/mL), MEAA exhibited 73.81%, 76.67%, 79.05%, 80.95% inhibition of denaturation of bovine serum, whereas at same concentrations, diclofenac sodium showed 90%, 95.71%, 97.14%, 98.57% inhibitions ($p < 0.001$). The results are represented in Fig. 3.

Effect of MEAA on membrane stabilisation by hypotonic induced haemolysis

The membrane stabilisation method is widely used to determine anti-inflammatory activity due to similarities in lysosomal membranes and human red blood cell

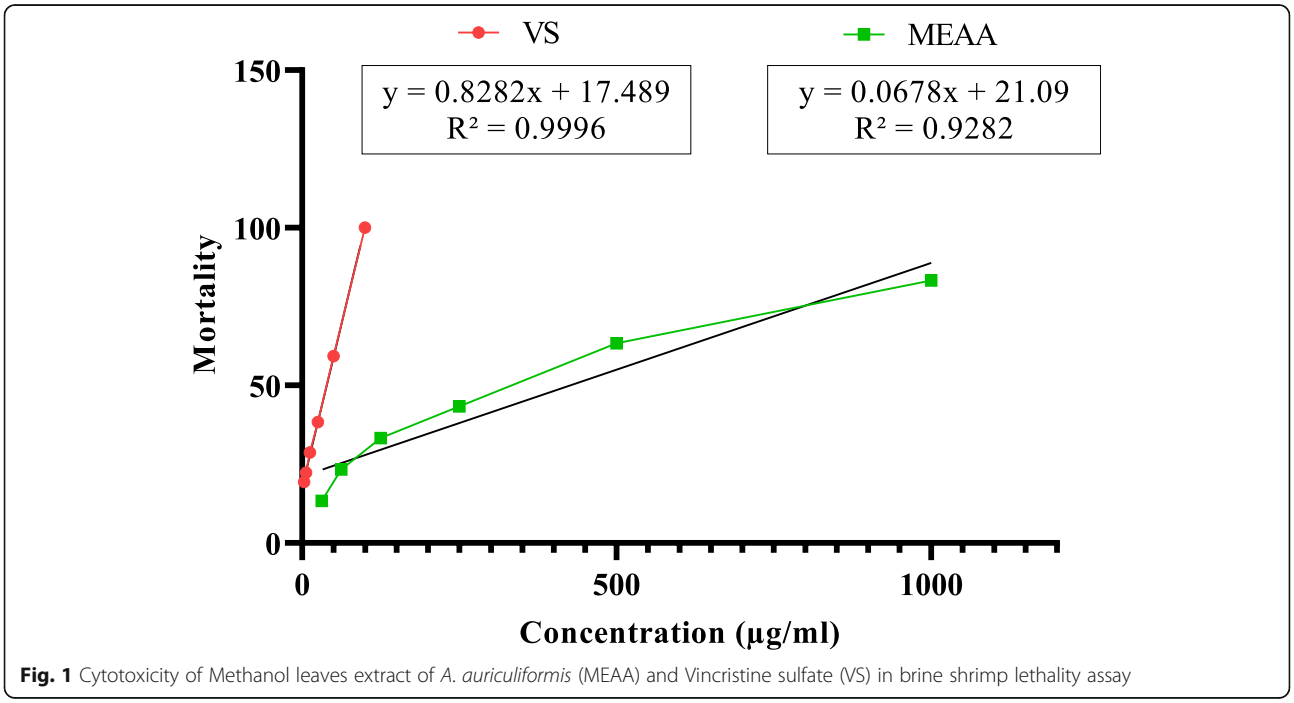

Fig. 1 Cytotoxicity of Methanol leaves extract of *A. auriculiformis* (MEAA) and Vincristine sulfate (VS) in brine shrimp lethality assay

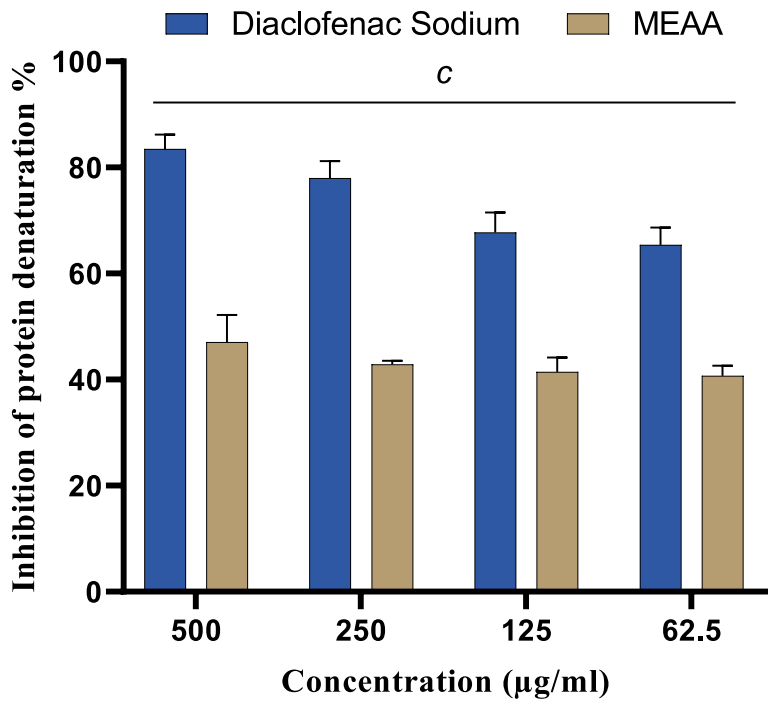

Fig. 2 Percentages of egg albumin protein denaturation by methanol extract of methanol leaves extract of *A. auriculiformis* (MEAA) and standard drug diclofenac sodium. Values are mean ± SEM ($n = 3$). $^{c}p < 0.001$, significantly different from control followed by Dunnett's test

membranes. Therefore, we also performed membrane stabilisation by hypotonic induced haemolysis method to further ascertain the anti-inflammatory activity of MEAA. In this experiment, different concentrations of MEAA were found to be dose-dependent and the percentage of haemolysis inhibition was significantly increased compared to the control mixture ($p < 0.001$). At different concentrations (31.25, 62.5, 125, 250, 500 and 1000 µg/mL), MEAA unveiled 63.39%, 73.63%, 77.95%, 81.98%, 86.78%, 89.88% of inhibition, whereas at same

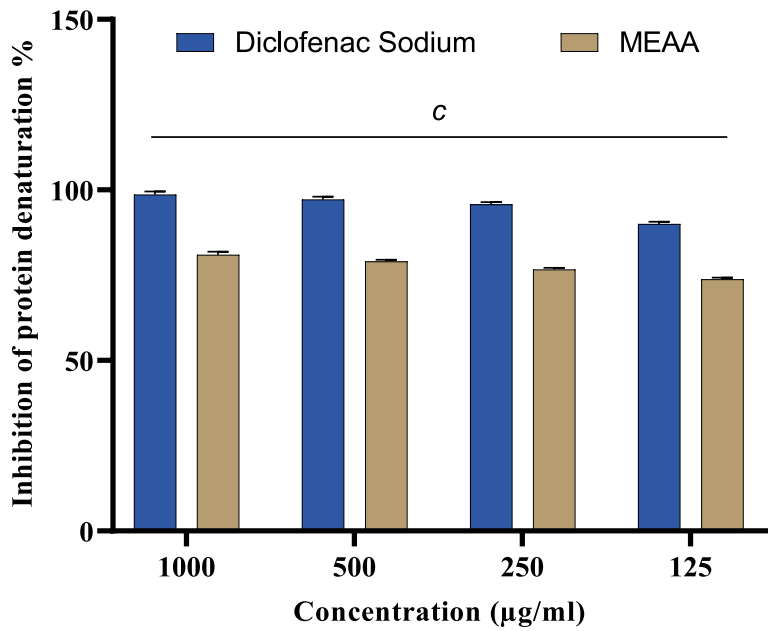

Fig. 3 Percentages of bovine serum albumin protein denaturation by methanol extract of methanol leaves extract of *A. auriculiformis* (MEAA) and standard drug diclofenac sodium. Values are mean ± SEM ($n = 3$). $^{c}p < 0.001$, significantly different from control followed by Dunnett's test

concentrations diclofenac sodium displayed 79.47%, 83.45%, 87.02%, 90.05%, 94.09%, 97.89% of haemolysis inhibition ($p < 0.001$). The result is summarised in Table 1.

Effect of MEAA on the worm *Tubifex tubifex*

Due to anatomical and physiological relevancy to intestinal worms (Annelida), *Tubifex tubifex* was used in our current experiment. Results have shown that the anthelmintic activity of MEAA was directly proportional to employed concentrations. From lowest to highest concentrations (5, 8 and 10 mg/mL), MEAA exhibited ($p < 0.001$) paralysis times of (26.03 ± 0.34), (20.67 ± 0.19) and (15.50 ± 0.04) minutes, while time of death were (59.65 ± 0.18), (50.18 ± 0.06), (45.14 ± 0.36) minutes respectively ($p < 0.001$). The positive control (levamisole, 1 mg/mL) exhibited paralysis time (3.22 ± 0.08) minutes and time to death of (6.19 ± 0.61) minutes ($p < 0.001$). Figure 4 outlines all the results.

Effect of MEAA on clot lysis

In this module, MEAA revealed significant clot lysis activity (36.42 ± 1.95%, $p < 0.001$), whereas standard control 100 μL of streptokinase unveiled (51.31 ± 5.14%, $p < 0.001$) compared to negative control water (6.62 ± 2.83%). All the data are displayed in Fig. 5.

Effect of MEAA on Brewer's yeast induced pyrexia mice model

Yeast-induced pyrexia model was used in our present study to identify fever relieving activity of MEAA. Statistical analysis showed that at both doses 200 and 400 mg/kg, percent of pyrexia inhibition was significantly dose-dependent. After 3 h of 200 mg/kg MEAA dose administration, pyrexia level in mice reduced significantly 49.05% ($p < 0.01$). The maximum antipyretic activity 21.23% was observed for dose 200 mg/kg, whereas the 400 mg/kg dose exhibited 15.18% inhibition. Standard drug paracetamol (150 mg/kg) revealed 78.67% inhibition. The result of the antipyretic test is documented in Table 2.

Table 1 Membrane stabilising activity of methanol leaves extract of *A. auriculiformis*

Diclofenac sodium		MEAA	
Concentration	% of inhibition	Concentration	% of inhibition
31.25	79.47 ± 1.07 ^{c}	31.25	63.39 ± 1.43 ^{c}
62.5	83.45 ± 1.09 ^{c}	62.5	73.63 ± 1.37 ^{c}
125	87.02 ± 0.63 ^{c}	125	77.95 ± 0.81 ^{c}
250	90.05 ± 1.42 ^{c}	250	81.98 ± 0.65 ^{c}
500	94.09 ± 0.86 ^{c}	500	86.78 ± 1.12 ^{c}
1000	97.89 ± 0.96 ^{c}	1000	89.88 ± 1.07 ^{c}

Values are expressed as mean ± SEM ($n = 3$); $^{c}p < 0.001$ are statistically significant comparison with diclofenac sodium followed by Dunnett's test

Molecular docking study for inflammation and pyretic

To perceive possible mechanism behind inflammatory and pyretic activity of *A. auriculiformis*, isolated selected compounds (2,4-ditert-butylphenol, 2-palmitoylglycerol, 3-hydroxy-β-damascone, α-monostearin) were docked with cyclooxygenase (COX)-1 (PDB ID: 2OYE), cyclooxygenase (COX)-2 (PDB ID: 6COX), mPGES-1 (PDB ID: 3DWW). For cyclooxygenase-1, 2-4-ditert-butylphenol revealed the best docking score (– 7.716 kcal/mol) bonded with the SER 530 (H-bonding), LEU 352, LEU 359, LEU 531, ALA 527 (Pi-alkyl-bonding) and with VAL 16, VAL 349 (Alkyl-bonding) pockets (Fig. 6). Whereas 2-palmitoylglycerol exhibited the lowest docking score (– 5.39 kcal/mol) and showed pocket bonding interactions with MET 522, SER 530 (H-bond); GLY 526 (van der waals); LEU 115, LEU 93, ALA 527 (Pi-alkyl-bonding); VAL 116, TYR 355, ILE 523, VAL 349, ILE 89 (Alkyl-bonding). The rank of binding scores were 2-4-ditert-butylphenol (– 7.716 kcal/mol) > 3-hydroxy-β-damascone (– 7.021 kcal/mol) > α-monostearin (– 5.6 kcal/mol) > 2-palmitoylglycerol (– 5.39 kcal/mol). Hence, 2,4-ditert-butylphenol also exhibited the best docking score for COX-2 receptor (– 6.954 kcal/mol) and α-monostearin showed the lowest binding score (– 4.072 kcal/mol). In this case, 2,4-ditert-butylphenol showed bonding interactions with HIS 90 (van der waals), LEU 531 (C-H bond); Leu 352 (Pi-alkyl-bonding), ALA 527 (Pi-alkyl-bonding), TYE 355 (Pi-alkyl-bonding), VLA 349 (alkyl-bonding), VAL 523 (alkyl-bonding) pockets and α-Monostearin showed LEU 352 (Pi-alkyl-bonding), ALA 516 (Pi-alkyl-bonding), PHE 518 (alkyl-bonding), HIS 90 (alkyl-bonding) pocket bonding interactions (Fig. 6). The ranking order of binding score for compounds with COX-2 receptor were 2,4-ditert-butylphenol (– 6.954 kcal/mol) > 3-hydroxy-β-damascone (– 6.882 kcal/mol) > 2-palmitoylglycerol (– 5.22 kcal/mol) > α-monostearin (– 4.072 kcal/mol). For mPGES-1 receptor, the best score was unveiled by 3-hydroxy-β-damascone (– 4.245). The pocket binding interactions are ASN 74 (H-bond and C-H bond), ARG 126 (H bond); ARG 73 (Pi-alkyl), ALA 31(Pi-alkyl); HIS 72 (alkyl), HIS 113 (alkyl), TYR 28 (alkyl). Minimum score exposed by 2-4-ditert-butylphenol (– 3.848) which bind at ALA 31 (H bond); HIS 72 (C-H bond); GLU 77 (Pi-Cation); ARG 126 (Pi-Anion); HIS 72 (Pi-Pi T-shaped). Conversely, 2-palmitoylglycerol and α-monostearin did not reveal any interaction with mPGES-1 receptor. All the results are shown in Table 3 and binding interactions displayed in Table 4.

Molecular docking for brine shrimp lethality bioassay

Human estrogen receptor (PDB ID: 1ERR) were used to perform molecular docking for brine shrimp lethality bioassay. Results show that 2-4-ditert-butylphenol gave the highest binding score (– 7.69 kcal/mol) (Fig. 6).

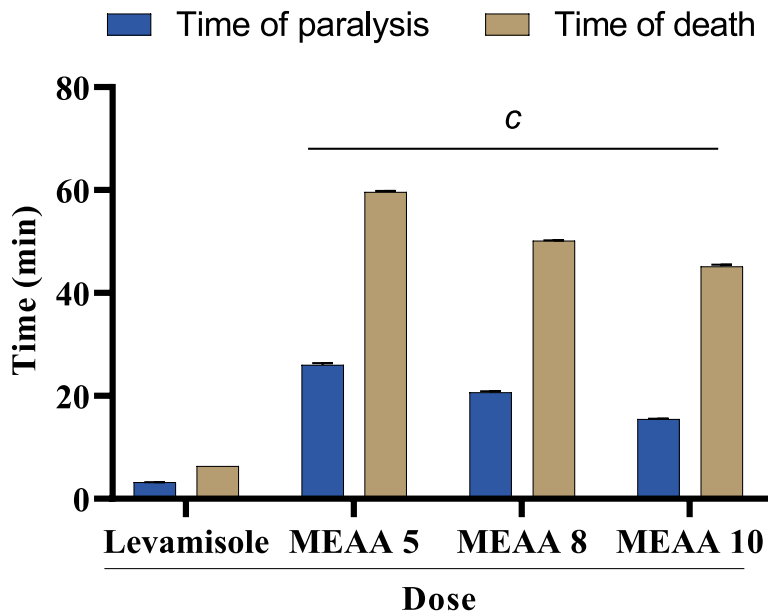

Fig. 4 Anthelmintic activity of methanol leaves extract of *A. auriculiformis* (MEAA). Values are represented in Mean ± SEM ($n = 3$). $^{c}p < 0.001$ statistically significant compared to levamisole followed by one-way ANOVA (*t* test)

Besides, 2-palmitoylglycerol gave the lowest binding score (− 4.241 kcal/mol) through one hydrogen bond to ASP 351. The binding score ranks are 2-4-ditert-butylphenol (− 7.69 kcal/mol) > 3-hydroxy-β-damascone (− 6.866 kcal/mol) > α-monostearin (− 4.948 kcal/mol) > 2-palmitoylglycerol (− 4.241 kcal/mol). All the results are shown in Table 3 and binding interactions displayed in Table 4.

Molecular docking for anthelmintic activity

Selected compounds are docked with the tubulin-colchicine receptor (PDB ID: 1SA0) for anthelmintic

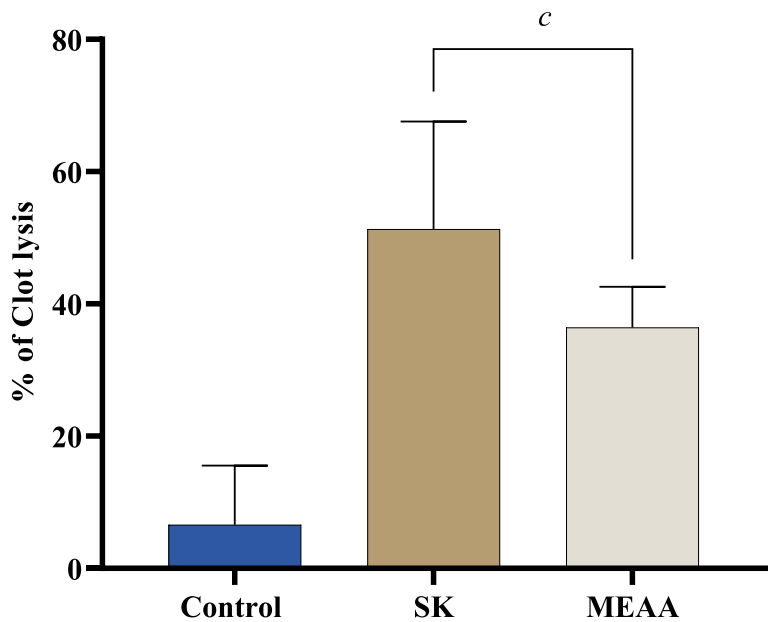

Fig. 5 Methanol leaves extract of *A. auriculiformis* (MEAA) and standard drug streptokinase (SK) effects on the clot lysis of blood. Values are represented in Mean ± SEM ($n = 6$). $^{c}p < 0.001$ were statistically significant in comparison with the negative control (water) followed by one-way ANOVA

Table 2 Antipyretic activity of methanol leaves extract of *A. auriculiformis* (MEAA) and standard drug

Dose	Initial temp (°C)	Temp of 18 h during pyrexia	1 h	2 h	3 h	4h
Control	37.24 ± 0.03	38.07 ± 0.27	38.15 ± 0.35	38.15 ± 0.23	38.09 ± 0.15	38.05 ± 0.25
Paracetamol	36.94 ± 0.28 [a]	38.55 ± 0.35 [a]	37.68 ± 0.17 [b]	36.85 ± 0.73 [b]	36.48 ± 0.33 [c]	36.41 ± 0.55 [c]
200mg/kg	37.09 ± 0.23 [a]	38.15 ± 0.35 [a]	38.07 ± 0.291 [a] (7.55%)	37.98 ± 0.27 [a] (16.04%)	37.63 ± 0.37 [b] (49.1%)	38.02 ± 0.2 [a] (12.3%)
400mg/kg	36.7 ± 0.37 [a]	38.61 ± 0.28 [a]	38.54 ± 0.267 [a] (3.66%)	38.35 ± 0.03 [a] (13.61%)	38.15 ± 0.08 [a] (24.08%)	38.24 ± 0.03 [a] (19.37%)

Values are expressed as mean ± SEM (n = 3); [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ significantly different in comparison with control. The data were analysed by ANOVA followed by Dunnett's test

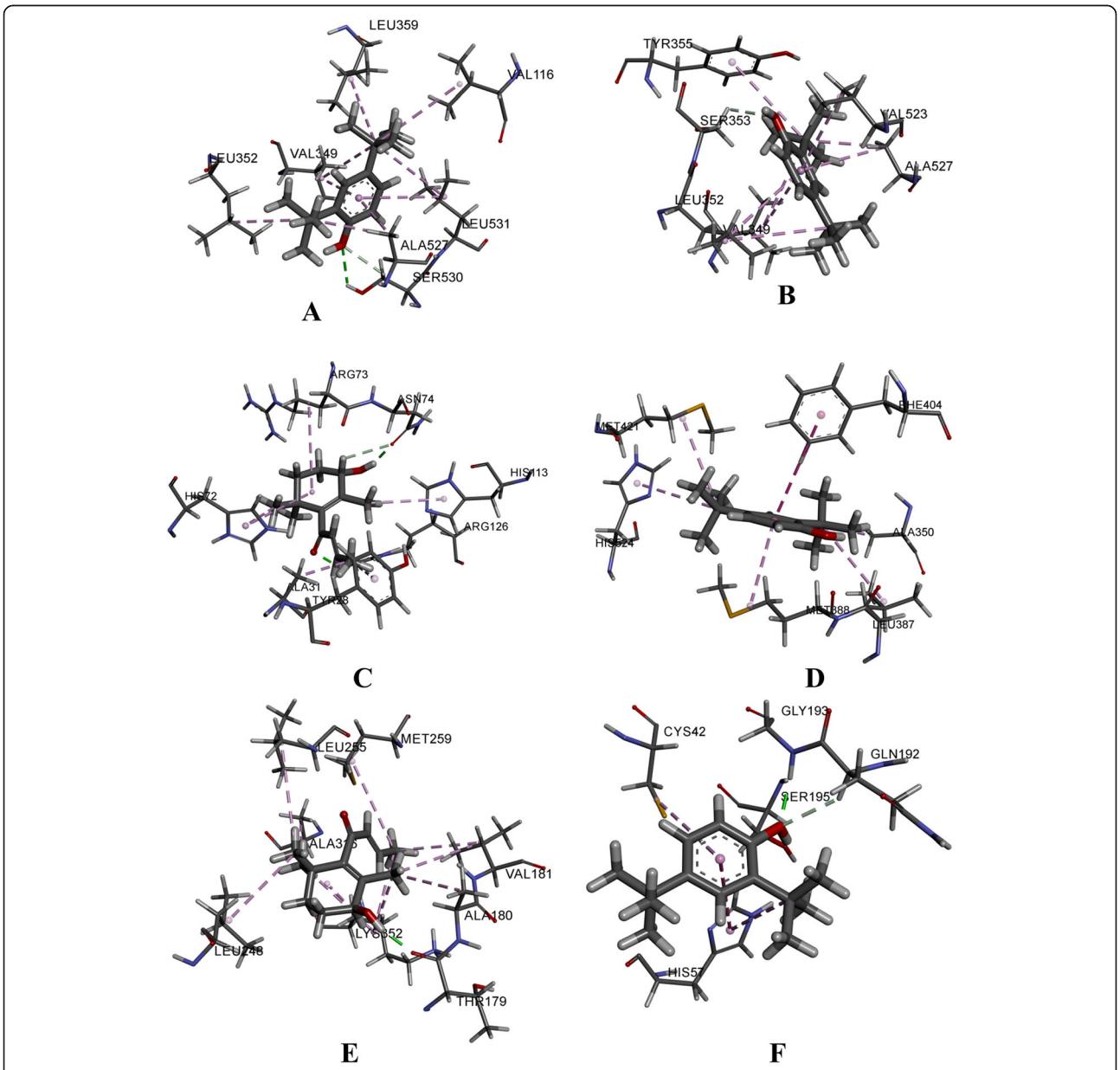

Fig. 6 3D representation of best docking score compounds. **a** 2,4-Ditert-butylphenol with COX1 receptor (PDB: 2OYE). **b** 2,4-Ditert-butylphenol with COX2 receptor (PDB: 6COX). **c** 3-Hydroxy-β-damascone with microsomal prostaglandin E synthase (mPGES)-1 (PDB: 3DWW). **d** 2-4-Ditert-butylphenol with human estrogen receptor (PDB: 1ERR). **e** 3-Hydroxy-β-damascone with tubulin-colchicine (PDB: 1SA0). **f** 2-4-Ditert-butylphenol with tissue plasminogen activator (PDB: 1A5H)

Table 3 Molecular docking score of isolated compounds from *A. auriculiformis*

Protein	Compounds	Docking score
2OYE	2-4-Ditert-butylphenol	– 7.716
	2-Palmitoylglycerol	– 5.39
	3-Hydroxy-β-damascone	– 7.021
	α-Monostearin	– 5.6
6COX	2,4-Ditert-butylphenol	– 6.954
	2-Palmitoylglycerol	– 5.22
	3-Hydroxy-β-damascone	– 6.882
	α-Monostearin	– 4.072
3DWW	2-4-Ditert-butylphenol	– 3.848
	2-Palmitoylglycerol	
	3-Hydroxy-β-damascone	– 4.245
	α-Monostearin	
1ERR	2-4-Ditert-butylphenol	– 7.69
	2-Palmitoylglycerol	– 4.241
	3-Hydroxy-β-damascone	– 6.866
	α-Monostearin	– 4.948
1SA0	2-4-Ditert-butylphenol	– 6.401
	2-Palmitoylglycerol	– 5.067
	3-Hydroxy-β-damascone	– 6.515
	α-Monostearin	– 5.077
1H5S	2-4-Ditert-butylphenol	– 5.42
	2-Palmitoylglycerol	– 4.269
	3-Hydroxy-β-damascone	– 3.494
	α-Monostearin	– 2.706

activity. The best docking fitness score shown by 3-hydroxy-β-damascone (– 6.515 kcal/mol) bonded to THR 179 pocket via H-bonding and with LEU 248, LEU 255, ALA 316, LYS 352, MET 259, ALA 180, VAL 181 pockets via alkyl-bonding (Fig. 6). Binding fitness score order are 3-Hydroxy-β-damascone damascone (– 6.515 kcal/mol) > 2-4-ditert-butylphenol (– 6.401 kcal/mol) > α-monostearin (– 5.077 kcal/mol) > 2-palmitoylglycerol (– 5.067 kcal/mol) as reported in Table 3. The binding interactions are shown in Table 4.

Molecular docking for clot lysis activity

The docking result for thrombolytic activity is documented in Table 3. In this assay, selected compounds are docked with tissue plasminogen activator receptor (PDB ID: 1A5H). The strongest docking interaction is exhibited by 2-4-ditert-butylphenol (– 5.42 kcal/mol) interacted with SER 195 (H-bonding), GLY 193 (H-bonding), GLN 192 (van der waals-bonding), HIS 57 (Pi-Pi T-shaped-bonding), CYS 42 (Pi-alkyl-bonding) pockets (Fig. 6). The lowest binding affinity was demonstrated by α-monostearin (– 2.706 kcal/mol). The other two compounds showed – 4.269 kcal/mol (2-palmitoylglycerol), – 3.494 kcal/mol (3-hydroxy-β-damascone) docking score, respectively. The binding interactions are documented in Table 4.

ADME/T screening for drug-likeness

Compounds compatibility as a drug molecule was investigated in the current study. According to Lipinski's 'Rules of Five', any orally administered compound must have molecular weight < 500 g/mol, hydrogen bond acceptor ≤ 10, hydrogen bond donor ≤ 5, lipophilicity value (LogP) ≤ 5. Two compounds (2-palmitoylglycerol, α-monostearin) in our study violated Lipinski's 'Rules of Five'. Further, the toxicological properties were predicted by using admetSAR online tool and confirm that none of the compounds possess carcinogenicity or AMES toxicity (Table 5). This elucidates that two derivatives (2,4-ditert-butylphenol and 3-hydroxy-β-damascone) can be considered as possible lead compounds and a good candidate for the advancement of new drugs.

Discussion

Brine shrimp lethality bioassay is known as a safe, economical and effective method to evaluate the bioactivity of plant products. Although an ideal biological response depends on bioactive constituents from plant parts but analysing plant crude extract is important. A good relationship has been found for pesticidal and cytotoxic activity between this method and solid human tumours which is beneficial for natural pesticides and pre-screening tests for antitumor research [19]. In this design, the higher the value of LD_{50}, the lower the toxicity of the extract is and vice versa [12]. In the current study, MEAA showed an LC_{50} value of 426.38 μg/mL, which is moderately toxic, and the standard control vincristine sulfate exhibited an LC_{50} value of 39.25 μg/mL. These moderate toxicity might be due to the presence of triterpenoid and saponins in *A. auriculiformis*; therefore, it can be used as an active chemopreventive agent previously suggested by Kaur et al. 2002 [37].

Traditionally, *A. auriculiformis* is recommended for rheumatism, pain, itching and allergy [38]. As these causes initiate by inflammation, therefore current investigation aimed to assess the anti-inflammatory activity of MEAA. Implicit drawbacks are associated with using animal models in pharmacological research, including lack of justifications and ethical concerns [20]. Therefore, in vitro protein denaturation and membrane stabilisation methods were proposed in the ongoing study to evaluate the anti-inflammatory property. In the living body, HRBC lysis (human red blood cell) and protein denaturation are often caused by inflammation, which initiates by the excess synthesis of COX [24]. During these conditions, proteins secondary and tertiary structures

Table 4 Binding interactions of isolated compounds with the respective proteins for different biological activities

Protein	Compounds	Binding Interaction	
		Hydrogen bond interactions	Hydrophobic bond interactions
2OYE	2-4-Ditert-butylphenol	SER 530 (H-bond)	LEU 352 (Pi-alkyl), LEU 359 (Pi-alkyl), LEU 531 (Pi-alkyl), ALA 527 (Pi-alkyl), VAL 16(Alkyl), VAL 349 (Alkyl)
	2-Palmitoylglycerol	MET 522 (H-bond), SER 530 (H-bond), GLY 526 (van der waals)	LEU 115 (Pi-alkyl), LEU 93 (Pi-alkyl), ALA 527 (Pi-alkyl), VAL 116 (Alkyl), TYR 355 (Alkyl), ILE 523 (Alkyl), VAL 349 (Alkyl), ILE 89 (Alkyl)
	3-Hydroxy-β-damascone		LEU 531 (Pi-alkyl), LEU 352 (Pi-alkyl), ALA 527 (Pi-alkyl), MET 522 (Pi-alkyl), TRP 387 (Alkyl), PHE 518 (Alkyl), ILE 523 (Alkyl), VAL 349 (Alkyl),
	α-Monostearin	ALA 527 (van der waals), MET 522 (van der waals), GLY 526 (van der waals)	LEU 92 (Pi-alkyl), LEU 93 (Pi-alkyl), ALA 527 (Pi-alkyl), ILE 523 (Alkyl), ILE 89(Alkyl), VAL 949 (Alkyl), VAL 116 (Alkyl)
6COX	2,4-Ditert-butylphenol	HIS 90 (van der waals), LEU 531 (C-H bond)	Leu 352 (Pi-alkyl), ALA 527 (Pi-alkyl), TYE 355 (Pi-alkyl), VLA 349 (alkyl), VAL 523 (Alkyl)
	2-Palmitoylglycerol	ASN 581 (H-bond) , GLN 192 (H-bond), HIS 351 (H-bond)	LEU 352 (Pi-alkyl), ALA 516(Pi-alkyl), PHE 518 (Alkyl), HIS 90 (Alkyl), VAL 523 (Alkyl)
	3-Hydroxy-β-damascone	TYR 355 (van der waals), ARG 120 (van der waals), LEU 359 (van der waals), GLY 526 (van der waals), MET 522 (van der waals), PHE 518 (van der waals), SER 353 (van der waals), SER 530 (H-bond)	LEU 352 (Pi-alkyl), ALA 527 (Pi-alkyl), LEU 531 (Pi-alkyl), TRP 387 (Alkyl), PHE 381 (Alkyl), LEU 384 (Alkyl), TYR 385 (Alkyl), VAL 349 (Alkyl), VAL 523 (Alkyl)
	α-Monostearin		LEU 352 (Pi-alkyl), ALA 516(Pi-alkyl), PHE 518 (Alkyl), HIS 90 (Alkyl)
3DWW	2-4-Ditert-butylphenol	ALA 31 (H bond), HIS 72 (C-H bond)	GLU 77 (Pi-Cation), ARG 126 (Pi-Anion), HIS 72 (Pi-Pi T-shaped)
	2-Palmitoylglycerol		
	3-Hydroxy-β-damascone	ASN 74 (H-bond and C-H bond), ARG 126 (H bond)	ARG 73 (Pi-alkyl), ALA 31 (Pi-alkyl), HIS 72 (Alkyl), HIS 113 (Alkyl), TYR 28 (Alkyl)
	α-Monostearin		
1ERR	2-4-Ditert-butylphenol		LEU 387 (Pi-alkyl), HIS 524 (Alkyl), MET 421 (Pi-alkyl), MET 388 (Pi-alkyl), PHE 404 (Pi-Pi T-shaped), ALA 350 (Pi-alkyl)
	2-Palmitoylglycerol	ASP 351 (H-bond)	LEU 525 (Pi-alkyl), LEU 346 (Pi-alkyl), LEU 387 (Pi-alkyl), ALA 350 (Pi-alkyl)
	3-Hydroxy-β-damascone		LEU 346 (Pi-alkyl), LEU 428 (Pi-alkyl), LEU 391 (Pi-alkyl), LEU 384 (Pi-alkyl), LEU 525 (Pi-alkyl), ALA 350 (Pi-alkyl), MET 388 (Pi-alkyl), MET 421 (Pi-alkyl), MET 343 (Pi-alkyl), PHE 404 (Alkyl), PHE 425 (Alkyl)
	α-Monostearin	ALA 350 (H-bond), ASP 351 (H-bond)	LEU 525 (Pi-alkyl), LEU 391 (Pi-alkyl), LEU 387 (Pi-alkyl), MET 343 (Pi-alkyl), MET 388 (Pi-alkyl), TRP 383 (Pi-Sigma), TYR 526 (Alkyl)
1SA0	2-4-Ditert-butylphenol		LEU 255 (Pi-Sigma), LEU 248(Pi-alkyl), ALA 250 (Pi-alkyl), ALA316 (Pi-alkyl), CYS 241(Alkyl)
	2-Palmitoylglycerol	LYS 254 (H-bond), GTP 600 (H-bond), LEU 248 (H-bond and van der waals), GLN 247 (van der waals)	LEU 255 (Pi-alkyl), LEU 242 (Pi-alkyl), ALA 354 (Pi-alkyl), ALA 316 (Pi-alkyl), LYS 352 (Alkyl), THR 353(Alkyl), CYS 241(Alkyl)
	3-Hydroxy-β-damascone	THR 179 (H-bond)	LEU 248 (Alkyl), LEU 255 (Alkyl), ALA 316 (Alkyl), LYS 352 (Alkyl), MET 259 (Alkyl), ALA 180 (Alkyl), VAL 181 (Alkyl),
	α-Monostearin	LEU 248 (H-bond), GTP 600(H-bond), ASN 249 (H-bond), LYS254 (H-bond), GLN 11 (H-bond and C-H bond)	LEU 255 (Pi-alkyl), ALA 180 (Pi-alkyl), ALA 354 (Pi-alkyl), ALA 316(Pi-alkyl), VAL 238 (Alkyl), TYR 202 (Alkyl), CYS 241(Alkyl), LYS 352(Alkyl)

Table 4 Binding interactions of isolated compounds with the respective proteins for different biological activities *(Continued)*

Protein	Compounds	Binding Interaction	
		Hydrogen bond interactions	Hydrophobic bond interactions
1H5S	2-4-Ditert-butylphenol	SER 195 (H-bond), GLY 193 (H-bond), GLN 192 (van der waals)	HIS 57 (Pi-Pi T-shaped), CYS 42 (Pi-alkyl)
	2-Palmitoylglycerol	GLU 60(H-bond and C-H bond), ARG 39 (H-bond)	CYS 42 (Alkyl), HIS 57 (Alkyl), TYR 99 (Alkyl), TRP 215 (Alkyl)
	3-Hydroxy-β-damascone	ASP 97 (C-H bond)	TYR 99 (Alkyl), ARG 174 (Pi-alkyl), TRP 215 (Alkyl)
	α-Monostearin	THR 175 (H-bond) THR 98 (H-bond) ASP 97 (C-H bond)	CYS 58 (Alkyl), CYS 42 (Alkyl), HIS 57 (Alkyl)

gone misplaced due to heat, strong acid or base, injurious agent, stress or organic solvent [10, 39]. In the current study, at both of protein denaturation model (egg albumin, BSA) MEAA exhibited a significant percentage of inhibition ($p < 0.001$), which was parallel to diclofenac sodium (standard drug). Besides, in membrane stabilisation assay, dose-dependent haemolysis inhibition of MEAA ($p < 0.001$) was found a comparative preventative response as close to the standard diclofenac sodium ($p < 0.001$). An earlier finding indicated the presence of flavonoids, tannins in *A. auriculiformis* [13]. Studies revealed that a number of flavonoids have strong inhibitory ability against the synthesis of cyclooxygenase and prostaglandin and several other enzymes such as phospholipase A2, protein kinase C, phosphodiesterases and protein tyrosine kinases [40, 41]. Besides, many investigations proposed plant metabolites like alkaloids, flavonoids, phenols and tannins possess anti-inflammatory activity due to their membrane stabilisation capabilities [20, 42]. Thus it can be presumed that due to the presence of flavonoids, tannins and phenols, *A. auriculiformis* possess significant anti-inflammatory activity. However, in-depth investigations by isolation and identification of bioactive metabolites from this plant extracts and fractions are highly recommended to reveal the exact pathway responsible for the aforementioned attributes.

Around 2 billion people all around the world suffer from parasitic infections. Parasitic worm infections are known as one of the major causes for developing many complicated chronic maladies like schistosomiasis, elephantiasis and onchocerciasis [43]. Though many synthetic agents are available for anthelmintic treatment but these agents produce severe complications. Therefore, due to less or fewer side effects in plant-derived, a new agent from a natural source has become an intense interest. *A. auriculiformis* is widely used as an anthelmintic in various regions of the world [13]. To verify this ethnopharmacological use, we planned an experiment on the methanol leaf extract of *A. auriculiformis* on the worm. In our current investigation, all concentrations provoked the worm's paralysis and caused death in a dose-dependent manner. Period of paralysis and death of worms were inversely proportional to extract concentrations ($p < 0.001$). Numerous studies advocated that phytochemicals like alkaloids, tannins, phenolic and flavonoids are responsible for anthelmintic activity [44]. Tannins might unveil activity by uncoupling oxidative phosphorylation and interacting with the worm's energy production, leading to the death of the worm. In contrast, alkaloid acts on the CNS of worms [44]. Previously, the presence of tannins has been reported from this plant [13]. Therefore, we can assume that, due to the ubiquity of tannins in *A. auriculiformis*, MEAA might interact with the glycoprotein of the parasite in the gastrointestinal tract and inhibit the worm's energy production which ultimately leads to the death of the worm.

Diverse assortments of thrombolytic agents are available, which clears the network of cross-linking fibrins via triggering the plasminogen enzyme. This process makes the clot soluble and renovates blood flow in blood vessels by clearing the clogged artery, thus avoid

Table 5 ADME/T and toxicological properties prediction of the bioactive compounds

Compounds	ADME/T					Toxicological properties			
Compounds	MW (< 500 g/mol)	HBD (< 5)	HBA (< 10)	LogP (< 5)	ROFV	Ames Toxicity	Carcinogens	AOT	ROT
2,4-Ditert-butylphenol	206.32	1	1	4.9	0	NAT	NC	III	2.2064
2-Palmitoylglycerol	330.5	4	2	6.1	1	NAT	NC	IV	1.0285
3-Hydroxy-β-damascone	208.30	3.7	1	2.02	0	NAT	NC	III	2.1347
α-Monostearin	358.6	4	2	7.4	1	NAT	NC	IV	0.1872

MW Molecular weight (acceptable range: ˂ 500 g/mol), *HBA* Hydrogen bond acceptor (acceptable range: ≤ 10), *HBD* Hydrogen bond donor (acceptable range: ≤ 5), *Log P* High lipophilicity (acceptable range, < 5), *ROFV* Rule of five violations, *NAT* Non-Ames toxic, *NC* Non-carcinogenic; Category III means 500 mg/kg > LD_{50} < 5000 mg/kg; Category IV means LD_{50} > 5000 mg/kg

permanent tissue damage. This therapy is useful to treat several conditions like myocardial infarction, deep vein thrombosis, thromboembolic strokes and pulmonary embolism. Streptokinase is one of that kind agent and used in our current study as a positive control group. In our investigation, MEAA disclosed significant ($p < 0.001$) clot lysis against the control group. Besides, Jia et al. 2016 hypothesised that a formulation of flavans and free B-ring flavonoids from this plant could be useful to inhibit platelet-induced thrombosis diseases [45]. Therefore, MEAA could be denoted as a potential source to inhibit blood clots in the future, although this outcome is not as significant as streptokinase.

Brewer's yeast-induced pyrexia model is known as a convenient test for screening antipyretic activity of synthetic drugs along with plant phytochemicals [26]. Yeast induces in the living body causes a biochemical cascade and increase the synthesis of cytokines such as tumour necrosis factor-α (TNF-α), IL-6, interferon-α and interleukin-1β especially prostaglandins. Increased synthesis of prostaglandins alters thermoregulation in the hypothalamus [46]. Therefore, any agent that can constrain this cascade of pyrexia-inducing mediators will be marked as an antipyretic agent (Paracetamol) [47]. In the current study, administration of MEAA (200 and 400 mg/kg) exhibited reduction of rectal temperature in rodents ($p < 0.05$). This reduction of temperature was observed might be due to the presence of some pharmacologically active constituents in *A. auriculiformis*, which interfere with the synthesis of prostaglandin. However, phytochemicals, specifically steroids and flavonoids, are known as a good antipyretic agent and in the previous study presence of these phytochemicals has been observed in *A. auriculiformis* [13, 48]. Nevertheless, it must be noted that diverse biochemical reactions take place during the synthesis of prostaglandins. Therefore, further in-depth investigation is worthwhile to regulate the exact biosynthetic pathway where the extract exerts fever relieving effects.

Computer-aided molecular docking helps to predict the molecular mechanism by ligand-target interactions and correlate the experimental data with a pharmacological response. This process plays a crucial role to bolster medical and pharmacological innovations by analysing the binding modes of active compounds against the targeted receptors. Therefore, in the present study, contemporary computer-aided molecular docking was done for previously isolated bioactive compounds via GC-MS analysis. Compounds were chosen based on Lipinski's 'Rules of Five' and further ADME/T properties were investigated. Against six targeted protein in the current study, 2-4-ditert-butylphenol unveiled promising score for inflammatory and pyretic (PDB ID: 2OYE, 6COX, 3DWW), cytotoxic (PDB ID: 1ERR) and thrombolytic (PDB ID: 1A5H) compared to other compounds. This outcome confirms that 2-4-ditert-butylphenol can directly bind with targeted enzymes and decrease enzymatic activities and reveal potential activity. Hence, in earlier, this compound expressed interaction with COX2, interleukin IL-6, IL-1b and TNF-α in RAW 264.7 cells and MCF-7 cell line activation of P53 gene has observed [49]. However, 3-hydroxy-β-damascone exhibited the best interaction score against helminthic (PDB ID: 1SA0), inflammatory and pyretic (PDB ID: 3DWW). Based on our current finding, we can conclude that due to the presence of these compounds, MEAA disclosed significant activity, and it could be a potential bioactive compound for treating several diseases.

Pharmacokinetic (ADME/T) and toxicological properties are imperative before the introduction of drugs in the commercial market. This step helps to understand the pharmacokinetic view and reduce cost during drug development. Therefore, selected compounds from methanol leaves extract of *A. auriculiformis* subjected to ADME/T analysis. Results exhibited that selected isolated compounds 2,4-ditert-butylphenol and 3-hydroxy-β-damascone satisfy Lipinski's 'Rule of Five' and indicate as a promising drug candidate for anti-inflammatory, antipyretic, anthelmintic and thrombolytic. In the toxicological study, selected compounds displayed non-Ames toxicity, non-carcinogenicity and none possess rat acute toxicity. Though two compounds α-monostearin and 2-palmitoylglycerol showed LD_{50} values more than 5000 mg/kg in acute oral toxicity, which means that these compounds are not orally safe. Thus, in silico results in this study referred all selected isolated compounds were accountable and similarities between biological and computer-aided models results were witnessed. Therefore, this investigation gives a better understanding via correlating with pharmacokinetics and pharmacodynamics properties with the experimental outcomes for next step investigation before the clinical trial.

Conclusion

This experiment confirms that methanol leave extract of *A. auriculiformis* has an interesting paradox having the potentiality to contribute to the development of new anti-inflammatory, antipyretic, anthelmintic and thrombolytic agents due to the presence of several secondary metabolites. In the computer-aided investigation, among all the previously isolated selected compounds, two compounds satisfied Lipinski's 'Rule of Five'. 2-4-Ditert-butylphenol unveiled significant binding interaction for anti-inflammatory, antipyretic and thrombolytic activities. Moreover, 3-hydroxy-β-damascone disclosed most

promising score for anthelmintic activity. In pharmacokinetic (ADME/T) and toxicological analysis, two compounds (2,4-ditert-butylphenol and 3-Hydroxy-β-damascone) revealed a safety profile with oral bioavailability from the druggable point of view. Therefore, comprehensive, in-depth analysis of this plant with isolated compounds is highly recommended to confirm the mechanisms of action for aforesaid pharmacological activities by the animal model and subsequently in humans for clinical efficacy.

Abbreviations

MEAA: Methanol extracts of *A. auriculiformis*; BSA: Bovine serum albumin; SK: Streptokinase; VS: Vincristine sulfate; s.c: Subcutaneous; BW: Bodyweight; ANOVA: Analysis of variance; SEM: Standard error of the mean; ADME/T: Absorption, Distribution, Metabolism, Excretion, and Toxicity

Acknowledgements

Authors are very much thankful to the Department of Pharmacy, International Islamic University Chittagong, Bangladesh for providing research facilities and other logistic supports.

Plant authentication

Acacia auriculiformis leaves were collected and identified from Rangamati region and authenticated by a taxonomist under the accession number Anwar-0870.

Authors' contributions

SN and MMH planned and designed the research. RK arranged the whole facilities for the research and supervised the whole research. SN, MMH and NI conducted the entire laboratory works with NBH and AMT. SN, NI, AMT and NBH imparted in study design and interpreted the results putting efforts on statistical analysis. The computational study carried out by MMH, AMT and SN. MMH, SN, AMT, NI and MAS participated in the manuscript draft and has thoroughly checked and revised the manuscript for necessary changes in format, grammar and English standard. MAS critically reviewed the manuscript. All authors read and agreed on the final version of the manuscript.

Funding

This work is conducted with the individual funding of all authors.

Availability of data and materials

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

Declarations

Ethics approval and consent to participate

The Swiss albino male mice of were procured from the Jahangirnagar University, Dhaka-1342, Bangladesh. The study approved by the Institutional Animal Ethical Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh according to governmental guidelines under the reference of Pharm/P&D/AEC-147/13-19. For ethical reasons, each animal was used only once and all animals were sacrificed at the end of the study.

Consent for publication

Not applicable.

Competing interests

The authors declared that they have no conflict of interest.

Received: 22 December 2020 Accepted: 4 March 2021
Published online: 20 March 2021

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