

Phytochemical profiling, anti-inflammatory, analgesic and antipyretic potentials of *Portulacaria afra* Jacq: Molecular docking of unexplored bioactive compounds

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Abstract

Natural products play an important role in novel drug discovery by providing structural leads for the pharmacotherapeutic agents against a variety of diseases. This study aimed to evaluate the possible, anti-inflammatory, antipyretic activity and analgesic potential of hydro-methanol extract (HMPA) of *Portulacaria afra* (acq) in a rat model to support its folk use. The ingredients in HMPA extract were identified by using GC/MS followed by molecular docking of 1-Coprosten-3-one semicarbazone, Phenol, 2, 2-methylenebis(7,9-ditert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione against Cyclooxygenase-1 and Cyclooxygenase-2 enzymes. The phytochemical test of HMPA extract showed a positive result for secondary metabolites of tannin, flavonoids, alkaloids, phytosterol, saponins, cardiac glycoside quinolones, triterpenoids, and carbohydrates. The HMPA extract showed a good concentration of total flavonoid content (163.25 ± 2.50 mg GAE/g) and total phenolic contents (153.21 ± 1.17 mg GAE/g). The antioxidant capacity of HMPA extract showed significant potential by DPPH, ABTS, and CUPRAC, FRAP methods. The Chromatogram by GC/MS showed compounds with anti-inflammatory potentials such as methyl octadeca-9, 12-dienoate, 9, 12, 15-Octadecatrienoic acid, palmitoleic acid, methyl palmitate, hexadecanoic acid, methyl ester, Parthenolide. In carrageenan-induced hind paw edema, assay results showed dose-dependent anti-inflammatory action. The different doses (100, 200, and 400 mg/kg) had anti-inflammatory activity in a dose-dependent manner. The analgesic activity by hot-plate, tail flicking, writhing test in rats HMPA extract demonstrated significant ($p < 0.05$) potential. The antipyretic activity was investigated by using yeast-induced pyrexia in albino rats. At the dose of 100 and 200, 400 mg/kg HMPA extract significantly decreased the temperature with maximum effect at the 3rd, 4th, and 5th h. The results of in vitro and in vivo experimental research confirmed the anti-inflammatory, analgesic, and antipyretic capabilities of *P. afra* and sustained folkloric claims of this plant.

Introduction

Inflammation autoimmune disease. Leukocytes, particularly neutrophils, are attracted to the inflammatory center in response to damage (Dawood et al., 2022). These is a natural protective response to tissue damage resulting from physical trauma, toxic chemicals, microbial agents, or even cells produce pro-inflammatory mediators such as cytokines (IL-1 β , IL-16), chemokines, prostaglandins which attract other inflammatory cells and establish effector functions to destroy infections (Basit et al., 2022). Prostaglandins are very effective vasodilators and they induce an inflammatory response as well as a fever (Sajid-Ur-Rehman et al., 2021a). Cytokines are endogenous pyrogens that cause fever and exert a negative effect on the progression of metabolic dysfunction (Shi et al., 2019).

The symptoms of inflammation are severe pain, swelling, loss of function of the affected area (Saleem et al., 2020). Standard anti-inflammatory treatments, such as non-steroidal anti-inflammatory medicines (NSAIDs) and corticosteroids, are used to treat moderate to severe pain and inflammations (Nguyen et al., 2020). Long-term usage of these NSAIDs inhibits prostacyclin and prostaglandin E₂, causing side effects such as nausea, vomiting, and peptic ulcer (Steiner and Higgins, 2018). There is a demand for alternative therapy for the treatment of infectious diseases that has few side effects from natural sources. Plant-derived compounds play a role in drug discovery and the development of new anti-inflammatory, analgesic, antipyretic medicines (Sajid-Ur-Rehman et al., 2021a).

Portulacaria afra is one of the dominant medicinally important plants widely grown in the east of South Africa belonging to the family Didiereaceae (Nakamura et al., 2021). In this moist climate, it is relatively rare, and tends to favor dryer rocky outcrops and slopes (Bruyns et al., 2014). *P. afra* is an annual succulent herb thick fleshy leaves for water storage (Iranshahy et al., 2017). This plant can grow up to 2 meters tall. It, has red stems with round fleshy leaves, and produces pink flower. It widely grows in warm climates in the eastern South Africa (De Vos, 2021). The genus *Portulaca* contains approximately 100 species with a wide distribution, primarily in tropical and subtropical regions, and a high degree of morphological variability (Borsai et al., 2018).

Portulacaria afra (*P. afra*) also known as "elephant bush," is a succulent plant in the Didereaceae family (Sajid-Ur-Rehman et al., 2021a). In a moist climate, it is comparatively infrequent and tends to favor dryer stony outliers and hills (Bruyns et al., 2014). *P. afra* leaves is used to treat a wide range of human pathologies, including skin diseases, respiratory problems, vomiting and diarrhea, inflammation, metabolic syndrome, and leukemia (De Wet et al., 2013). The plant extracts seem to have antibacterial and anti-diabetic effects (Olaokun et al., 2017, Nciki, 2015). As previously mentioned, genus *Portulaca* produces important phytochemicals with high Possibility as drug candidates for a wide range of targets. Though there hasn't been a lot of research into potential bioactivities for this plant, so there's a gap in scientific knowledge about its complete potential. The current study aims to scientifically validate the folklore use of *P. afra* by conducting analgesic, anti-inflammatory, antipyretic experiments in rat models.

Materials And Methods

Collection of Plant

Plant material was collected in March 2019 from the Baghdad-ul-Jadeed campus of The Islamia University of Bahawalpur, Pakistan. The plant was identified and specified voucher number 314 was allotted by taxonomist Ghulam Sarwar, Assistant professor department of Botany, The Islamia University of Bahawalpur, Pakistan. The plant was dried, mangled, pulverised, and stored in glass jar.

Extraction Method

Dried powder of plant material (2kg) was soaked in 80% hydro-alcoholic (methanol) solvent for 3days at room temperature. It was agitated regularly during maceration. After 3 days, the plant material was passed through muslin cloth and then filtered by using Whatman-1 filter paper. This procedure was repeated twice to collect all the remaining phytochemicals. All collected filtrates were dried using a rotary evaporator at 35 °C under less pressure. The dried crude extract was weighed and stored in an airtight container for further analysis.

Chemicals and Drugs

Indomethacin from Shanxi Guangsheng pharmaceutical, carrageenan (Sigma–Aldrich, USA) provided diclofenac sodium and paracetamol from Agron Remedies Pvt. Ltd. while tramadol was purchased from Searle Pharma- ceuticals, Indomethacin (15mg/kg), diclofenac sodium (15 mg/kg), and paracetamol 50 mg/kg and different doses of HMPA extract (100, 200,400mg/kg) were prepared in double-distilled water for administration in rats and mice.

Phytochemical Screening, Flavonoids, and Total Phenolic Contents

Phytoconstituents tests were used to screen out a variety of secondary metabolites (Hegde et al., 2015). The total phenolic contents (TPC) of HMPA extract was determined using a colorimetric Folin–Ciocalteu reagent (FCR) technique with slight modifications. The 10 µl of diluted FCR (10%) mixed with 100 µl of sample solution, 90 µl of 15% w/v aqueous sodium carbonate solution. This mixture was incubated for 90 minutes at 37 °C and absorbance was measured at 750 nm. The results were reported as milligram gallic acid equivalents per gram of dry extract (Sajid-Ur-Rehman et al., 2021b).

An aluminum chloride colorimetric test was used to assess the total flavonoid content (TFC), and quantity of total flavonoids was reported rutin equivalents (mgRE/g extract) (Zengin et al., 2015). A calibration curve with a range of 0–100 µl (0–100 mg) was created using rutin as the standard. The 25 µl of 1% sodium nitrite solution and 100 µl of test solution was mixed and stand for 5 minutes then added 10 µl of 1% aluminum chloride solution again stand for five

minutes Afterwards 36 µl of 4% of sodium hydroxide was mixed and was diluted by using methanol (28 µl) and at 510 nm, absorbance was read. The calibration curve was used for the calculation of TFC and expressed as milligram rutin equivalent per gram of dry extract (mg of RE/g extract).

Determination of Antioxidant Potential

Antioxidant activity of HMPA extract was assessed by using two radical scavenging assays i.e., DPPH, ABTS

While reducing power was determined by using i.e., FRAP, CUPRAC assay reported protocol by (Asif et al., 2016, Khurshid et al., 2019).

Gas Chromatography-Mass Spectroscopy (GC–MS) Analysis

The phytoconstituents of HMPA extract were determined using GC MS analysis (Agilent, 6890 series Hewlett Packard, 5973) mass choosy detection systems, with HP-5MS column (250 m in diameter, 30 m in length 0.25 m in film thickness). The temperature at the inlet was 220 °C. The oven temperature steadily increased from 60 °C to 280 °C at a rate of 3 °C/min. The carrier gas was pure helium gas (99.995 percent purity) flowing at a rate of 1 mL/min in constant flow mode. 1.0 L of prepared extracts diluted with individual solvents were injected in a split-less mode. The compound was identified using a NIST library search (NIST 14).

Experimental animals

Wistar albino rats weighing 150–230 g were kept in the animal house of the Pharmacology & Physiology research laboratory at IUB Punjab, Pakistan. All animals used in the study were housed in polycarbonate cages that measured (47 × 34 × 18 cm³). The typical conditions of temperature (25 ± 2 °C) and humidity (50–55%), as well as exposure to 12 hour light and dark cycle, were maintained throughout the study. The animals were fed conventional animal food and given free access to water. To reduce animal stress, acclimatized to the test environment for one week before the trial began. All experimental protocols were reviewed by the Pharmacy Animal Ethics committee with approval no PAEC22/73 in the Faculty of Pharmacy, IUB Punjab, Pakistan.

Physio-pathological Observations

Physiological and pathological investigations were made on albino mice (25–30 g) kept under normal circumstances. Mice were arbitrarily separated into five groups of six mice and starved overnight before the experimental assay, which was supplied with only water. Animals were given HMPA extract at escalating doses of 0.5, 1, 3, 5, and 10 g/kg/oral by stomach intubation, whereas the control group was given a 10% DMSO solution in distilled water. The animals were intensively monitored for 48 hours and then for 14 days. Animal behavior such as convulsion, tremor, salivation, writhing reflex, and behavior pattern was detected, as well as usual unwanted effects such as diarrhea and weight loss (Javed et al., 2020).

Acute Anti-inflammatory Activity

Carrageenan Induced hind-paw edema model was used with minor modification to determine *in vivo* anti-inflammatory potential of HMPA extract. Wistar albino rats have fasted with free access to water, and animals were divided into five groups (n=6). All groups received 0.1 mL freshly prepared 1% carrageenan injected into the right hind paw except control (Sajid-Ur-Rehman et al., 2021a). The digital Vernier caliper (Mitutoyo, Japan) was used to measure the paw size of

rats. The rats were given a vehicle (distilled water 5 ml/kg, i.p), HMPA extract (100, 200, and 400 mg/kg, i.p) and standard indomethacin (15 mg/kg, i.p) half an hour earlier. The paw edema of all above-mentioned groups was measured just before and after carrageenan injection at "0 h," and subsequently at 1, 2, 3, and 4 hours. The previously reported formula was used to compute the % inhibition of paw edema ([Javed et al., 2020a](#)).

$$\text{Percentage Inhibition} = \frac{\text{Control mean} - \text{treated mean}}{\text{Control Mean}} \times 100$$

Antipyretic Activity

Wistar albino rats have fasted with free access to water, and animals were divided into five groups of six animals each. Before induction of pyrexia, the rectal temperature of all animals was measured using a digital clinical thermometer. All animals were injected in the back neck with a 15 % w/v of Brewer's yeast (10 ml/kg aqueous solution) to induce pyrexia. Rectal temperature was measured after 18 hours, and animals with a temperature increase of at least 0.5 °C were included in the study. The pyretic animals were treated orally with HMPA extract (100, 200, and 400 mg/kg) and standard paracetamol (PCM 50 mg/kg orally) distilled water (5 mg/kg, orally to control group). The rectal temperature of all animals was measured at the 1st, 2nd, 3rd, and up to the 6th hour following HMPA extract administration by putting a digital clinical thermometer 2 cm into the rectum ([Javed et al., 2020a](#))

Analgesic Activity

Analgesic activity of HMPA extract was measured by using tail immersion method, hot plate method, Acetic acid-induced writhing method. Wistar albino rats (100 – 190 g) were divided into six groups each group having six animals. To control group 10% DMSO (5 ml/kg) was given orally, to the standard group diclofenac sodium (15 mg/kg) and tramadol (30 mg/kg) was given orally. All animals were treated with HMPA Extract (100,200,400 mg/kg) orally.

Tail Immersion Method

The tail Immersion method was performed with minor modifications as described([Sajid-Ur-Rehman et al., 2021b](#)). For measuring tail-flick response animals were divided into five group and each group having six animals. In this study, a thermostat was set to keep the water temperature between 50± 55°C. The rat tail (3cm) was immersed in hot water, and the reflexes of the tail retracting from the hot water were recorded before and after dose administration in rats. Following that, the experimental animals were administered HMPA extract and standard drug and recorded the reaction time again at 30, 60, 90, and 120 minutes.

Hot Plate Method

The hot-plate test was performed with minor modifications as described by ([Sajid-ur-Rehman et al., 2021c](#)).The HMPA extract and standard drug was given orally to the respective groups after one hour of dose administration the individual rat was placed on a hot plate (VELP®, Italy) set to 50–55 °C.

The response time of rats was recorded in seconds by observing licking, lifting, or jumping before and after the therapy at 30, 60, 90, and 120 minutes. To avoid paw injury, 25 seconds was set as the cut-off time

Acetic acid-induced writhing method

According to (Sajid-Ur-Rehman et al., 2021a) the acetic acid-induced writhing model was used with minor modification. For this experiment, albino rats (100–150 g) were divided into five groups (n=6). The control group got DW 5 ml/kg while the standard group received diclofenac sodium 15mg/kg whereas the treatment group got HMPA extract in various doses (100, 200, and 400 mg/kg) orally. 30 minutes after treatment 0.6 % v/v acetic acid (0.1 mL) was administered orally. The numbers of abdominal muscle contractions (writhes) were counted for 5 minutes.

Molecular docking analysis

The chemical compounds got from the results of GCMS analysis were subjected to molecular docking with some anti-inflammatory protein targets. The docking analysis used the crystal structures of the Cyclooxygenase 1 (PDB: 4O1Z) and Cyclooxygenase 2 (PDB: 5IKV) protein targets from the PDB (<https://www.rcsb.org/>). Polar hydrogens were inserted into the protein. Water, inhibitors, and extra chains were removed from the protein and then converted into the PDBQT format. To analyze their potential against the proteins, the ligands were taken from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). The ligands were input into Open Babel and used the PyRx application to reduce their energy. The compounds were then transferred to PDBQT format for further investigation. The grid box is then constructed in the desired dimensions. Finally, the Discovery studio visualized the interactions.

Statistical Analysis

Result values were represented in mean $\pm$ SEM (n = 6). Two-way ANOVA followed by a test tokay's was performed using Graph Pad Prism (San Diego, CA, USA) software. A p-value of less than 0.05 was considered statistically significant.

Results

Preliminary Phytochemicals

Secondary metabolites such as saponins, alkaloids, phenols, tannins, and glycosides were detected in HMPA extract.

Estimation of Total Phenolic Contents (TPC)

Total phenolic content and flavonoid contents of HMPA extract were calculated as (159.21 $\pm$ 2.22 mgGAE/g) and (163.25 $\pm$ 2.50 mgGAE/g) of dry extract respectively.

Determination of Antioxidant Potential

The HMPA extract showed significant antioxidant potential through the CUPRIC method and moderate antioxidant potential through DPPH, FRAP, ABTS method (fig.1).

GC–MS profiling

The existence of plant metabolites with documented anti-inflammatory and analgesic potential was exposed by GC–MS analysis as indicated in Fig. 2 and Table 1.

Table 1

GC-MS analysis of HMPA extract (compound identification was based on the NIST library)

Peak number	RT (min)	Area	Name of Compound	Molecular Formula	Molecular mass	Biological Activity
Peak -1	3.07	5.99	Ethylbenzene	C ₈ H ₁₀	106.16	n/a
Peak -2	3.13	41.65	1,4-Dimethylbenzene	C ₈ H ₁₀	106.16	n/a
Peak -3	3.37	17.92	o-Xylene	C ₈ H ₁₀	106.16	n/a
Peak -4	10.19	1.64	Heneicosane	C ₂₁ H ₄₄	296.6	antimicrobial activity(Vanitha et al., 2020)
Peak -5	10.43	2.42	Phenol,2,4-bis(1,1-dimethylethyl)-, phosphite	C ₁₇ H ₃₀	278.5	Antifungal(Ren et al., 2019)
Peak -6	11.29	0.72	1-Hexadecene	C ₁₆ H ₃₁ Br	224.42	Antimicrobial, antioxidant (Mushtaq et al., 2013)
Peak -7	12.63	1.46	Heptacosane	C ₂₇ H ₅₆	380.7	Antibacterial (Konovalova et al., 2013)
Peak -8	13.50	0.74	Dichloroacetic acid	C ₁₇ H ₃₄	128.94	Antifungal and antioxidant (Sympli and Bioinformatics, 2021)
Peak -9	15.06	2.74	Hexadecanoicacid, methyl ester	C ₁₈ H ₃₆ O ₂	270.5	Antioxidant(Sympli, 2021)
Peak -10	15.11	1.51	7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	C ₁₇ H ₂₄ O ₃	276.4	Antimicrobial activity(El-Fayoumy et al., 2021)
Peak -11	17.26	1.33	methyloctadeca-9,12-dienoate	C ₁₉ H ₃₄ O ₂	294.5	Anti-inflammatory, hypocholesterolemic, cancer preventive, antiarthritic, antihistaminic (Krishnamoorthy and Subramaniam, 2014)
Peak -12	17.35	1.67	9,12,15- Octadecatrienoic acid	C ₁₈ H ₃₀ O ₂	278.4	Ant-inflammatory, antihistamine, anticornory, antiacne antimicrobial, antioxidant(Kumar et al., 2010)
Peak -14	23.42	8.27	1-Coprosten-3-one semicarbazone	C ₂₈ H ₄₇ N ₃ O	441.7	Antibacterial Activity(Hariri et al., 2016)
Peak -15	32.96	10.34	1,3-Bis(trimethylsilyl)benzene	C ₁₂ H ₂₂ Si ₂	222.47	Antioxidant, antibacterial (Mou et al., 2013)

Physio-pathological Observations

Physiological and pathological tests in mice using an increasing dose of HMPA extract up to 10 mg/kg were carried out and presented safe. There was no evidence of toxicity in terms of behavioral abnormalities or mortality after 48 hours.

Carrageenan-induced hind paw edema

The HMPA extract showed anti-inflammatory effect at various doses i.e. 100, 200, 400 mg/kg. At 100 mg/kg HMPA extract showed anti-inflammatory activity after 3rd and 4th hour i.e. (3.03±0.46 mm, 3.77±0.75 mm) respectively. At 200 mg/kg HMPA extract showed anti-inflammatory activity after 2nd, 3rd, 4th hour comparable to standard i.e. (3.09±0.4 mm, 3.96±0.87 mm, 3.00±0.32 mm). While at 400 mg/kg HMPA extract showed anti-inflammatory activity greater than standard after 1st, 2nd, 3rd, 4th hour i.e. (3.43±0.15 mm, 3.25±0.09 mm, 3.10±0.23 mm, 2.81±0.14 mm) fig.3.

Antipyretic Activity

HMPA extract significantly reduced temperature at doses of 100 and 200, 400 mg/kg, with the greatest effect at the 4th and 5th hour of treatment (Fig.4).

Analgesic Activity

Hot-plate Method

At doses of 100, 200 and 400 mg/kg, the HMPA extract demonstrated significant ($P < 0.05$) pain dormancy at various time intervals (30, 60, 90, and 120 minutes). During all observations tramadol significantly reduced pain (fig.5)

Tail Flicking Method

To measure analgesic activity, the tail immersion method was performed. The HMPA extract at 100, 200, and 400 mg/kg exhibited significant ($p < 0.05$) results in pain latency when compared to control group. The HMPA extract (Figure 6).

Acetic acid-induced writhing method

HMPA extract at (100, 200, 400 mg/kg) showed dose-dependent reduction in acetic acid-induced writhes.

The effect of HMPA extract was compared with standard control (fig.7)

Molecular docking analysis

Molecular docking was performed to explore the synergistic effect of the GC-MS-identified compounds on COX-1, COX-2 inhibition. A high binding affinity was observed for 1-Coprosten-3-one semicarbazone, Phenol, 2,2'-methylenebis, 7,9-ditert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione as shown in table (2 and fig.8)

Table 2

Binding affinities of HMPA extract *Pafra* against COX-1 and COX-2 targets.

Compounds Name	COX-1			COX-2		
	Binding affinity	Amino acids	Types of interaction	Binding affinity	Amino acids	Types of interaction
1-Coprosten-3-one semicarbazone	-8.3	LEU A:295	Alkyl Pi alkyl	-8.2	SER A:119	H bond CH bond
		VAL A:291	Alkyl		VAL A:89	Alkyl
		LEU A:408	Alkyl H bond		ILE A:112	Alkyl H bond
		HIS A:207			LEU A:108	Pi alkyl
		HIS A:446			TYR A:115	
Phenol,2,2'-methylenebis	-7.7	PRO A:153	Pi alkyl H bond	-7.2	ARG A:469	Pi alkyl CH bond
		CYS A:41	Pi sulfur CH bond		LYS A:468	Pi alkyl H bond
		TYR A:39	Pi alkyl H bond		HIS A:38	Pi T shaped
		ARG A:469	Donor-Donor			
		GLN A:42				
		LYS A:468				
7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	-7.1	LEU A:115	Alkyl	-6.6	PRO A:156	Alkyl
		ILE A:89	Alkyl			
		VAL A:116				

Discussion

The current study was planned to explore the pharmacological potential of HMPA extract to scientifically prove its folkloric uses. In this study, the phytochemical investigation was carried out by using guidelines mentioned in the

phytochemistry manual (Hegde et al., 2015). Our results exhibited a positive result for secondary metabolites of tannin, flavonoids, alkaloids, phytosterol, saponins, cardiac glycoside quinolones, and triterpenoids. Flavonoids and phenolic compounds present in this species might be related to performing anti-inflammatory action by acting on various pathways, i.e. scavenging oxidative radicals, may act on nuclear factor kappa (NF- κ B) activity, or inhibiting COX2 enzymatic reactions.

In the present study, HMPA extract demonstrated significant scavenging capabilities using the DPPH and ABTS techniques. The FRAP chemical is being used to alleviate messy free radicals by contributing an electron to them, while the CUPRAC test is a redox interaction between the CUPRAC reagent and antioxidants found in the plant extract. This plant's antioxidant capability suggested the existence of phenolic compounds with a lot of hydroxyl groups (Lekouaghet et al., 2020). The HMPA extract showed significant antioxidant potential in the CUPRAC assay. In DPPH, FRAP, ABTS assays, HMPA extract showed moderate antioxidant potential. Our results are steady with the previous finding in which antioxidant activity was less by the DPPH method (Olaokun et al., 2017, Khanyile et al.). Total antioxidant data exposed that HMPA extract has a high potential for antioxidant action that might help to prevent inflammation caused by free radicals.

The genus *Portulaca* has been shown to have anti-inflammatory properties by suppressing pro-inflammatory cytokine levels (Baradaran Rahimi et al., 2019, Iranshahy et al., 2017). Certain natural fatty acid esters, such as methyl palmitate, have inhibitory capacity against NF- κ B, which is accompanied by the downregulation of inflammatory channels (Palu et al., 2004). The existence of components as analyzed by GC-MS analysis also reinforced the plant's capability for anti-inflammatory, analgesic, and antipyretic potential. For the first time, the HMPA extract was investigated by GC-MS analysis. The existence of fifteen compounds with varying retention durations, chemical formulas, and molecular weight was identified i.e., 1,3-dimethyl-7-propylidene, benzene1, 3-dimethyl-7-propylidene (1.26%) Ethylbenzene (5.99%), P-Xylene (41.65%), O-Xylene (17.92%), Heneicosane (1.64), Phenol,2,4-bis (1,1-dimethyleth (2.42%), Hexadecanoic acid methyl ester (2.74%), 7,9-ditert-butyl-1-oxaspiro, 6,9-diene-2,8-dione (1.51%) methyloctadeca-9,12-dienoate (1.33%), 9,12,15-octadecatrienoic acid (1.67%), Coprosten-3-one semicarbazone (8.27%) Table.1.

The chemical compound 9, 12, 15-Octadecatrienoic acid is a fatty acid that contains hydroxyl group existing in HMPA extract have anti-inflammatory properties due to reduction of PGE2, NO, and COX-2, which are achieved by suppression of MAPK activation and histone acetylation (Guerrero et al., 2017). Phenol,2,2'-methylenebis was identified from HMPA extract present in several plants is traditionally used for its anti-inflammatory and analgesic properties. Numerous experimental studies have shown that they have anti-inflammatory characteristics due to their ability to disrupt the NF- κ B pathway (Shaukat et al., 2021). Hexadecanoic acid, methyl ester was a main compound in the HMPA extract which has antioxidant, antidiabetic (Ahmadi et al., 2021), anti-inflammatory properties, as well as the potential to lower blood cholesterol (Kaizal and Hussein, 2019). Our results are consistent with the previous results on the same genus which were reported by (Alu'datt et al., 2019). Naturally produced fatty acid esters, such as methyl palmitate, have inhibiting capability against NF- κ B, resulting in the enzyme inhibition of the inflammatory channel (Mantawy et al., 2012, El-Demerdash, 2011, Palu et al., 2004). Phenol, 2,4-bis (1, 1-dimethyl ethyl) have antimicrobial (Salini et al., 2014) antifungal (Rangel-Sánchez et al., 2014) antioxidant potential (Ghalloo et al., 2022). The presence of constituents as determined by GC-MS analysis also reinforced the plant's promising anti-inflammatory, analgesic, and antipyretic actions.

In the ongoing investigations *in-vivo* studies were performed to determine the anti-inflammatory, antipyretic, analgesic potential of HMPA extract. Physio-pathological examinations validated the vast safe zone for HMPA extract revealing it's no toxicity profile. Carrageenan is a potent molecule that promotes the production of pro-inflammatory and inflammatory mediators (leukotrienes, prostaglandins, bradykinin, histamine, TNF (Javed et al., 2020b, Posadas et al., 2004). Prostaglandins are the primary cause of acute inflammation. Even though both the cyclooxygenase and lipoxygenase pathways are important in the inflammatory process (Nunes et al., 2021). As a result, it was suggested that

the HMPA extract's induced anti-inflammatory potential might be related to one or more inflammatory mediators such as histamine, bradykinin, or prostaglandin leukotrienes.

Fever is caused by PGE₂ activation of the cyclooxygenase enzyme in the preoptic zone of the hypothalamus (Sajid-Ur-Rehman et al., 2021a). Cytokines in the hypothalamus stimulate the cyclooxygenase COX-2 to produce prostaglandin E₂ (PGE₂), which is thought to be the primary downstream mediator of fever (Nguyen et al., 2020, Yam et al., 2018). In the current research, the body rectal temperature of rats (98.08±0.30) was measured using a digital thermometer before the administration of the HMPA extract. At the dose of 100 and 200, 400 mg/kg HMPA extract significantly decreased the temperature with maximum effect at the 3rd, 4th, and 5th hour. Notably, the later effect was similar to the standard antipyretic drug, paracetamol (fig.4). After the 24th hour, the rectal temperature of rats was normalized as compared to standard. The HMPA extract showed antipyretic effect might be due to inhibition of prostaglandin synthesis; however, studies need to be done to validate its mechanism, which might be either central inhibition or a disruption in the pyrexogenesis stage, which could link peripheral inflammation with central PGE₂ production (Malvar et al., 2014).

Analgesic activity of HMPA extract was assessed using the tail flicking, hot-plate, and acetic acid-induced writhing method. Results showed that HMPA extract exhibited a significant analgesic effect ($p < 0.05$) at the doses of 100, 200, and 400 mg/kg. The HMPA extract analgesic efficacy could be attributed to its capacity to reduce prostaglandin formation via inhibiting cyclooxygenase activity (Rahmatullah et al., 2013). It is clear from the reported data that supraspinally mediated analgesic activity is mediated by μ , κ receptors whereas spinal analgesic action is mediated by δ κ μ opioid receptors (Angst and Clark, 2006). The peripheral pain feeling is caused by a localized inflammatory response caused by the release of arachidonic acid from tissue phospholipids via the COX pathway (Gawade, 2012). As a result, the findings of this study showed that the analgesic activity of HMPA extract may be attributed to central action on opioid receptors and via suppression of opioid receptors.

The computational technique of molecular docking is currently widely used in drug development. Docking gives the advantage of evaluating the binding affinity of protein binding complexes. In present study the chemical components from GCMS, as well as indicating the kind of interaction of the research compounds at the enzyme or receptor's location via particular critical interactions (Chandak et al., 2014). PyRx is a virtual screening tool that performs flexible multiple ligand docking analyses within protein binding sites (Borges et al., 2018). A total fifteen compounds docked against the Cyclooxygenase 1 (PDB: 4O1Z), Cyclooxygenase 2 (PDB: 5IKV). The hydrogen bond is important in protein-ligand interactions, and other hydrophobic interactions such as alkyl, pi alkyl, and so on also provide persistent binding of ligands to proteins (Chen and Oezguen, 2016). The best RMSD values were found with meloxicam and flurbiprofen for the corresponding protein targets COX-1 and COX-2. We have identified three compounds having the best energy affinity against these two proteins. With Cox-1 and COX-2, 1-Coprosten-3-one semicarbazone has the lowest binding affinity (-8.3 and -8.2 respectively), alkyl and pi sigma, and hydrogen bond interactions with the amino acids. It's worth noting that the 1-Coprosten-3-one semicarbazone also had a lot of interactions with amino acids near to the COX-1 and COX-2 active site's amino acids. As a result, this molecule may alter the local structure, which may have biological impacts. The other two compounds Phenol, 2,2'-methylenebis, and 7,9-ditert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione have -7.7 and -7.1 binding affinities while Phenol, 2,2'-methylenebis has two hydrogen bonds with CYS A:41 and GLN A:42 while 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione has hydrophobic interactions with different amino acids. Phenol, 2,2'-methylenebis and 7,9-ditert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione have -7.2 and -6.6 binding affinities with COX-2 while Phenol, 2,2'-methylenebis showed hydrogen bond but 7,9-ditert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione showed alkyl interaction.

Conclusion

The present study reveals the phytochemical analysis, antioxidant, analgesic, anti-inflammatory, and antipyretic potential of HMPA extract in rats. It can be concluded that HMPA extract has dose-dependent anti-inflammatory, analgesic, and antipyretic effects due to the presence of phenolic and flavonoids, as well as phytocompounds such as 9, 12, 15-octadecatrienoic acid, palmitoleic acid, hexadecanoic acid, methyl ester, and methyl palmitate, that were identified through GC–MS analysis. Molecular docking analysis is a structure-based drug design approach. The HMPA compounds 1-Coprosten-3-one Phenol, 2, 2-methylenebis, 7, 9-ditert-butyl-1-oxaspiro [4.5] deca-6, 9-diene-2, 8-dionesemicarbazone, exhibited high binding affinity with atomic energy -8.3, -7.7, -7.1 kcal/mol and showed excellent docked compounds against cox-1 and cox-2. The current research article signifies the first report on anti-inflammatory, analgesic, and antipyretic effects, GC–MS analysis, and molecular docking HMPA extract. So the findings of this study indicated that this plant will be an excellent candidate for the treatment of inflammation and pain-related illnesses.

Declarations

Author Contributions

Sobia Tabassum Performed the experimental work and wrote the manuscript.

Saeed Ahmad: Planned the project. Barkat Ali: Elucidation of the GC–MS results. Kashif-ur-Rehman Khan¹: Proof reading M. Sajid-ur-Rehman¹: Manuscript formatting. Muhammad Jafar Iqbal Statistical analysis. Fouzia Tabassum: Authentication of plant with voucher number. GS: Identification and collection of plant. Bilal Ahmad Ghalloo: Animal handling. Faisal Usman, Maqsood Ahmad: Help in experimental work. All authors carefully read and approved the manuscript.

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conflict of interest

The authors declare no conflict of interest.

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Figures

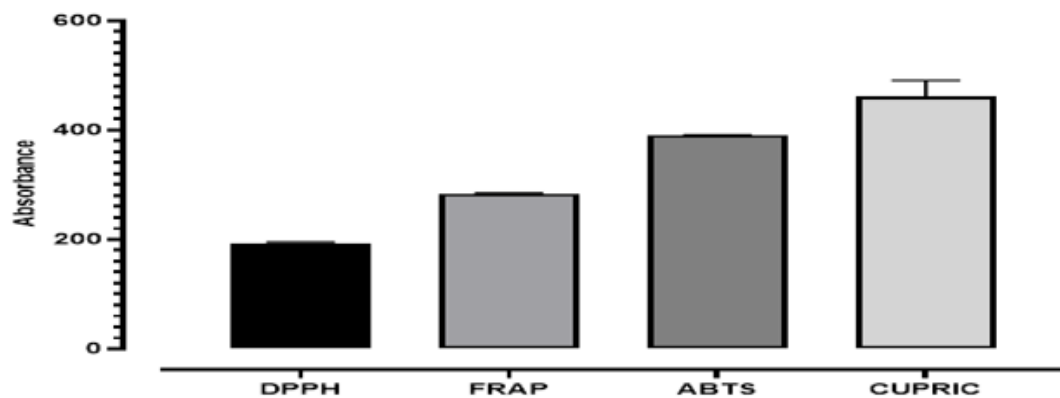


Figure 1

The anti-oxidant potential HMPA extract of *P. afra*. All values are expressed in mean $\pm$ SD and all procedures were carried out thrice. Results of DPPH, ABTS, CUPRAC, and FRAP are expressed in (mgGAE/g).

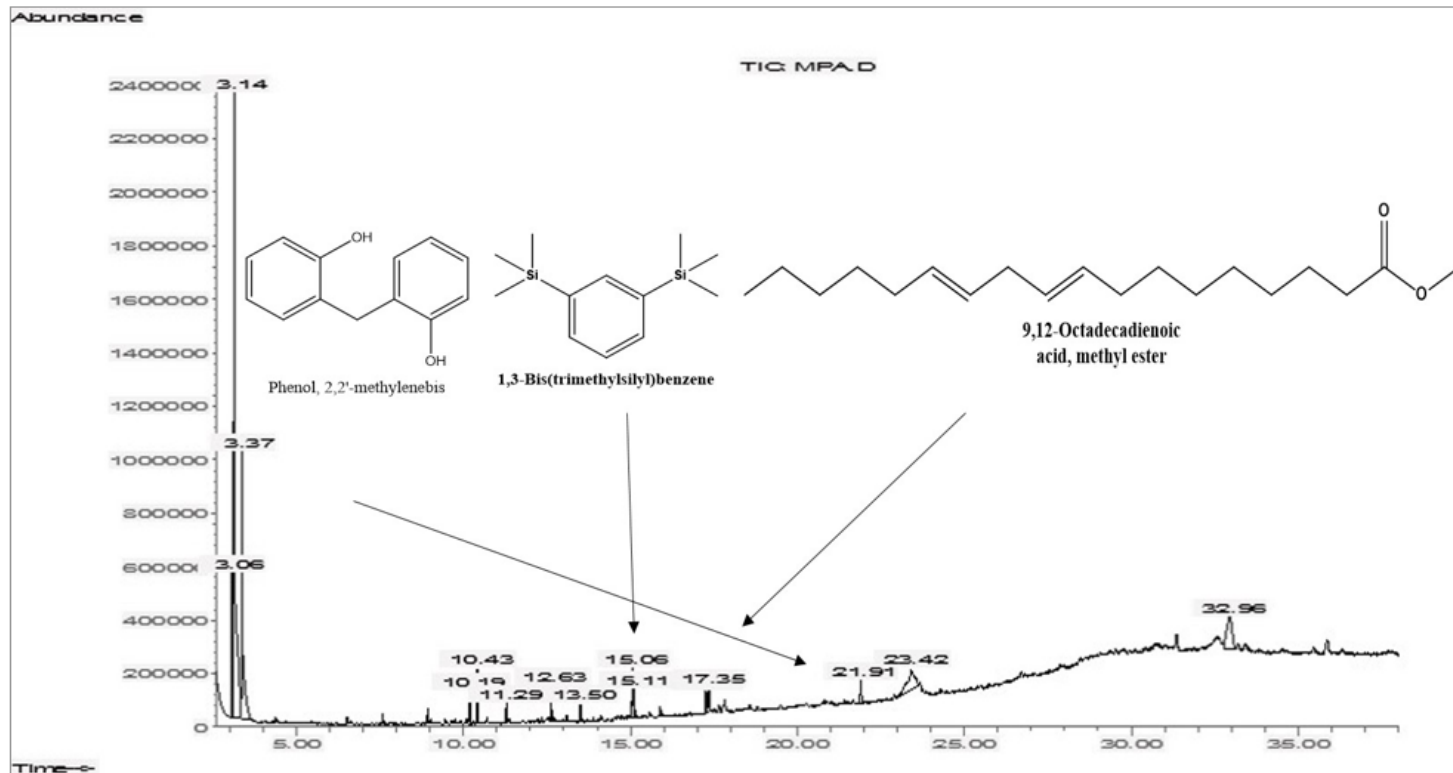


Figure 2

GC-MS Spectra of HMPA extract (compound identification was based on the NIST library).

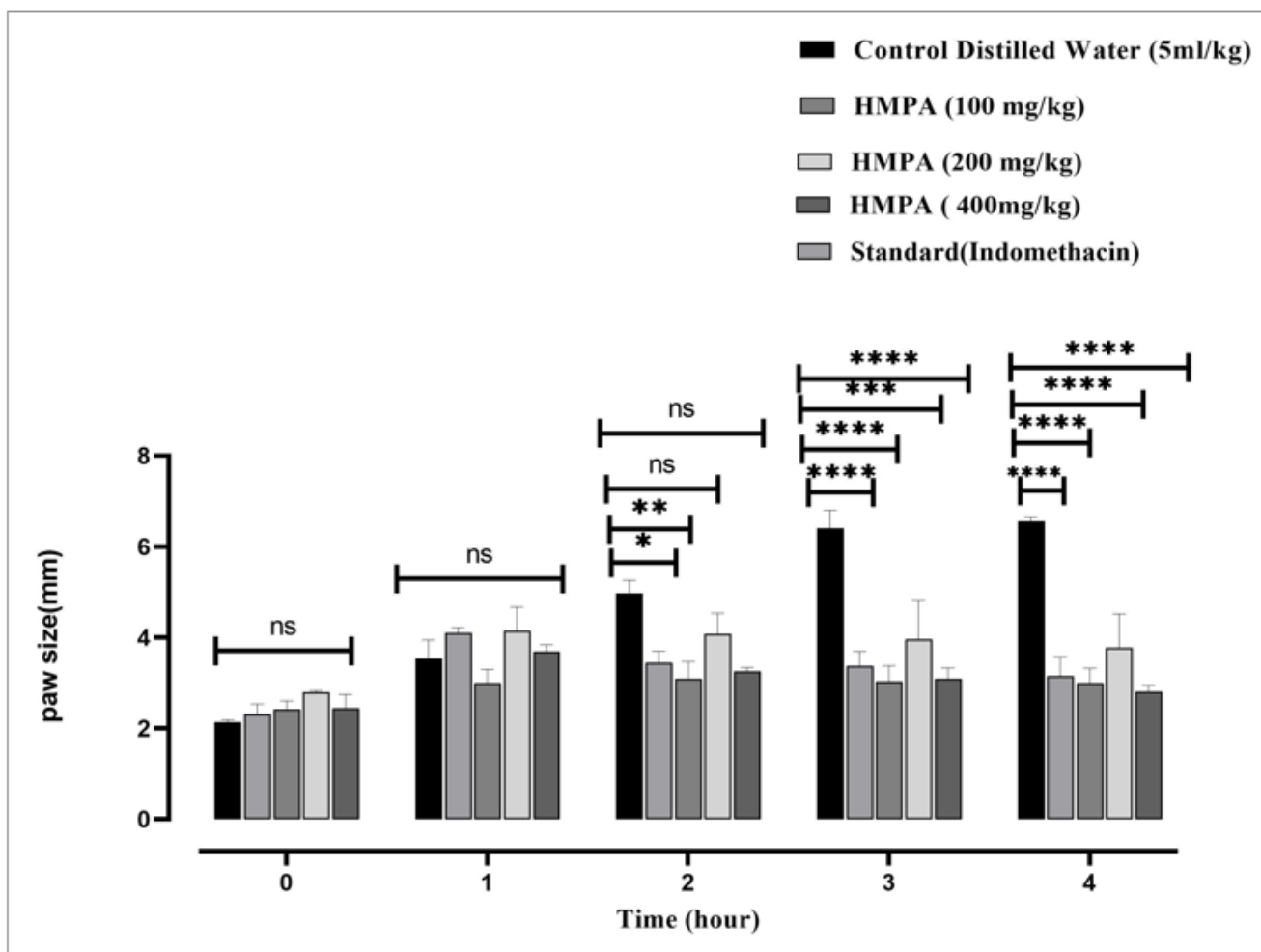


Figure 3

In the carrageenan-induced inflammation assay, paw diameter was significantly reduced in a dose-dependent manner. All values are given in mean $\pm$ SEM. N = 6. P < 0.001 (***) and P < 0.05 (*) as compared to the control (Two-way ANOVA followed by Tukey test).

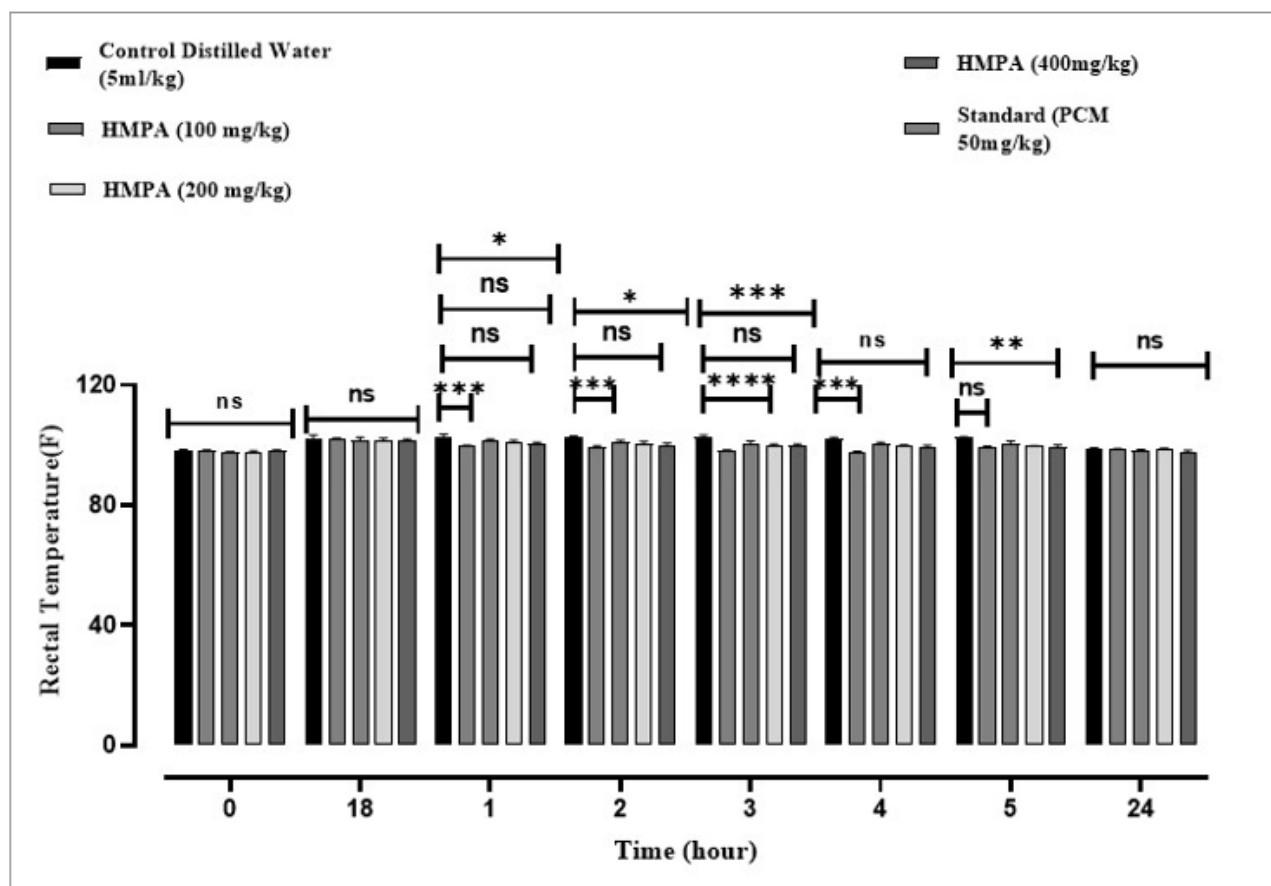


Figure 4

The antipyretic effects of HMPA extract and paracetamol (PCM), using yeast-induced pyrexia test in Wistar albino rats. All values are given in mean $\pm$ SEM. N = 6. P < 0.001 (***) and P < 0.05 (*) as compared to the control (Two-way ANOVA followed by Tukey test).

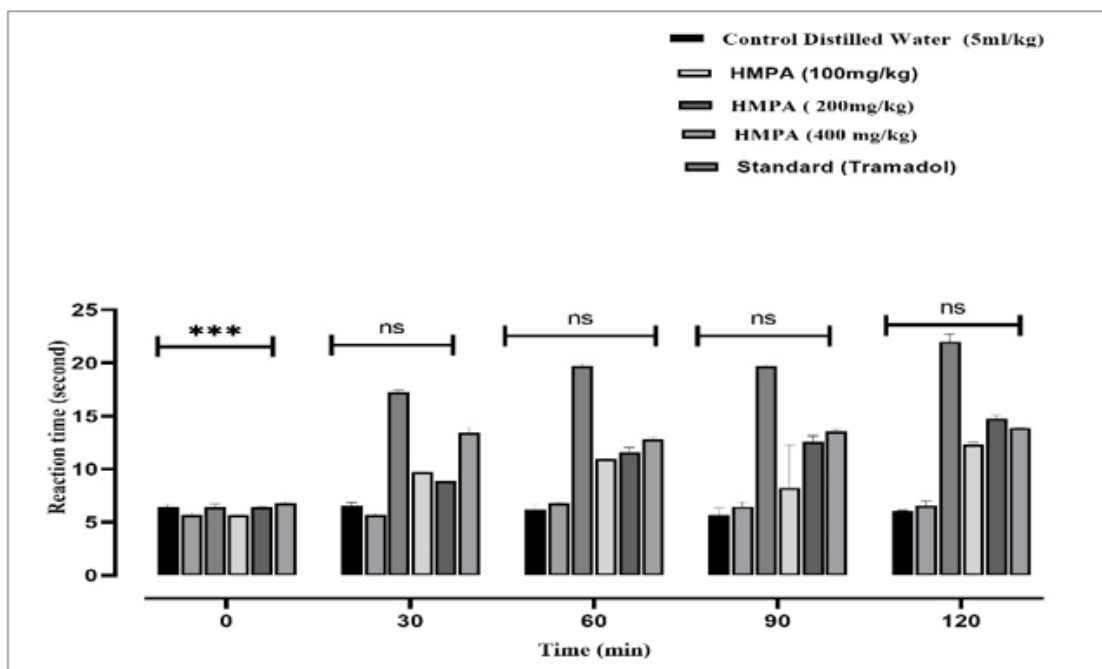


Figure 5

In the hot-plate test, different doses of HMPA extract and tramadol drug increased latency time. All resulting values are mentioned in mean± SEM. n=6. p <0.001 (***) and p< 0.05 (*) when compared to the control group.

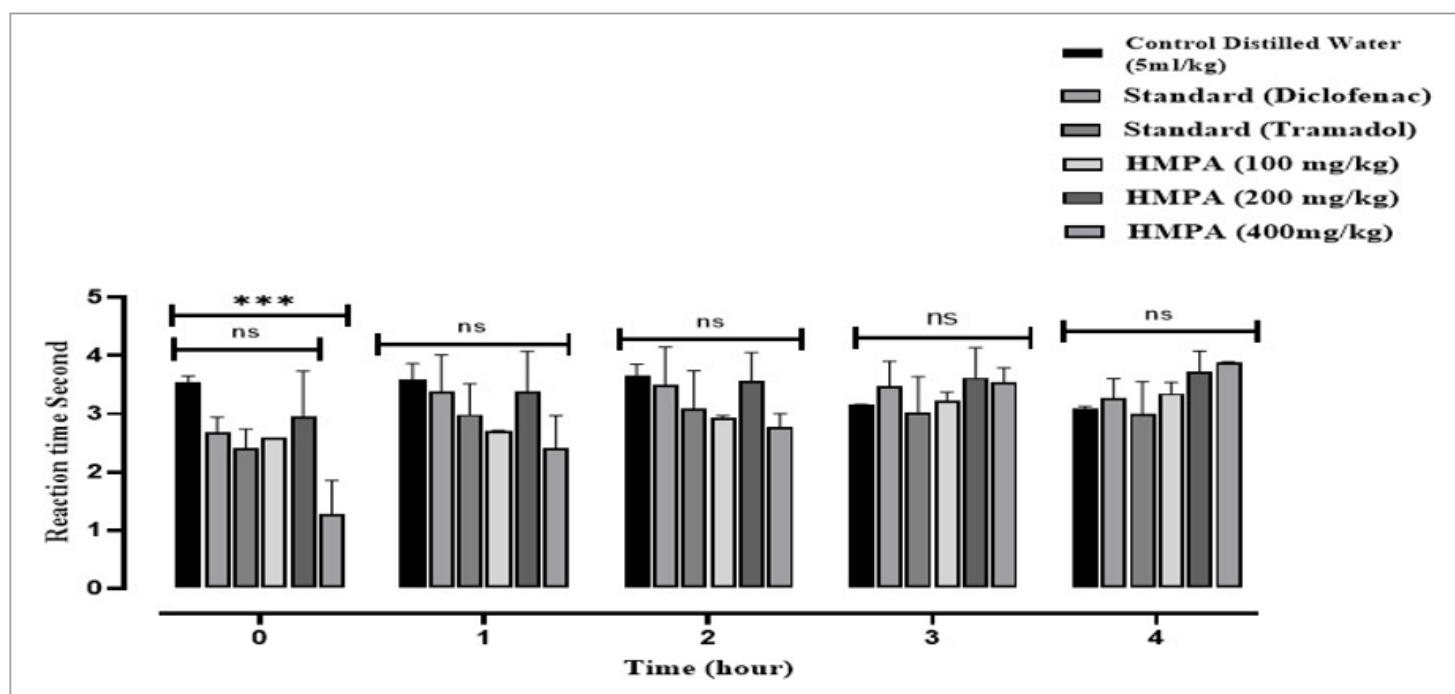


Figure 6

There was an increase in latency time with different doses of HMPA extract, diclofenac, and tramadol medication in the tail flicking test. All results are shown in mean ± SEM. n=6 P< 0.001 (***) and p< 0.05 (*) when compared to the control

group.

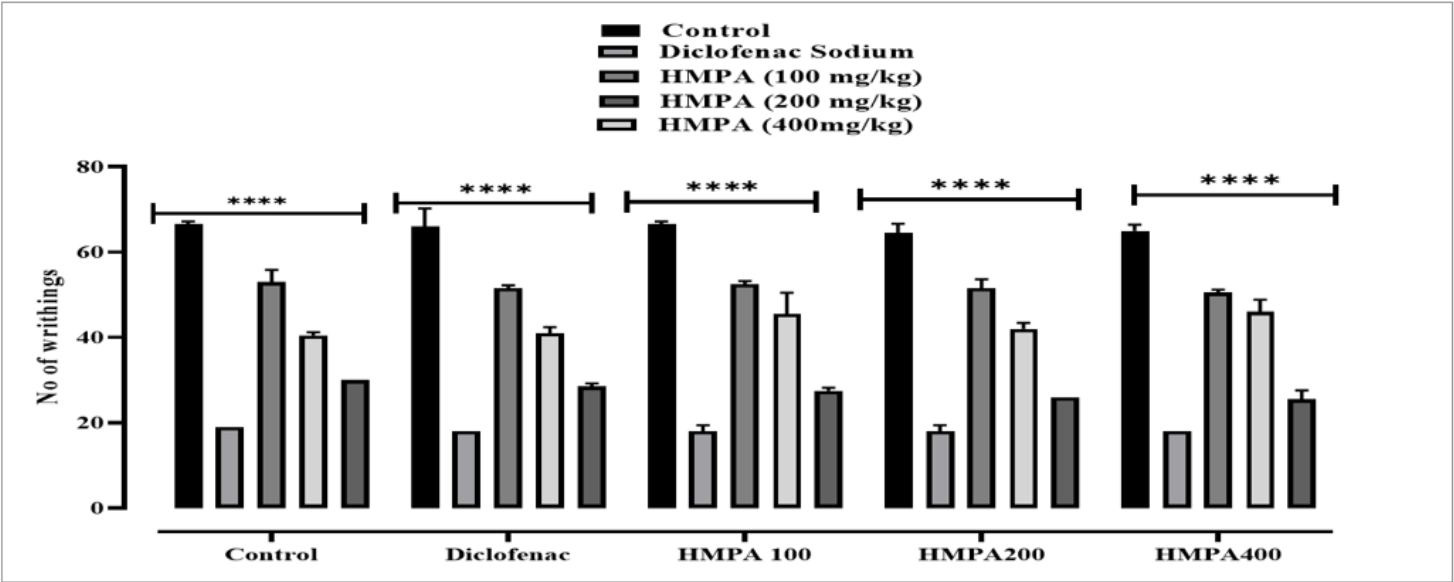


Figure 7

In acetic acid-induced writhing test HMPA extract and diclofenac sodium drug reduced the writhing response. All resulting values are mentioned in mean±SEM. n=6. p<0.001 (***) and p< 0.05 (*) when compared to the control group.

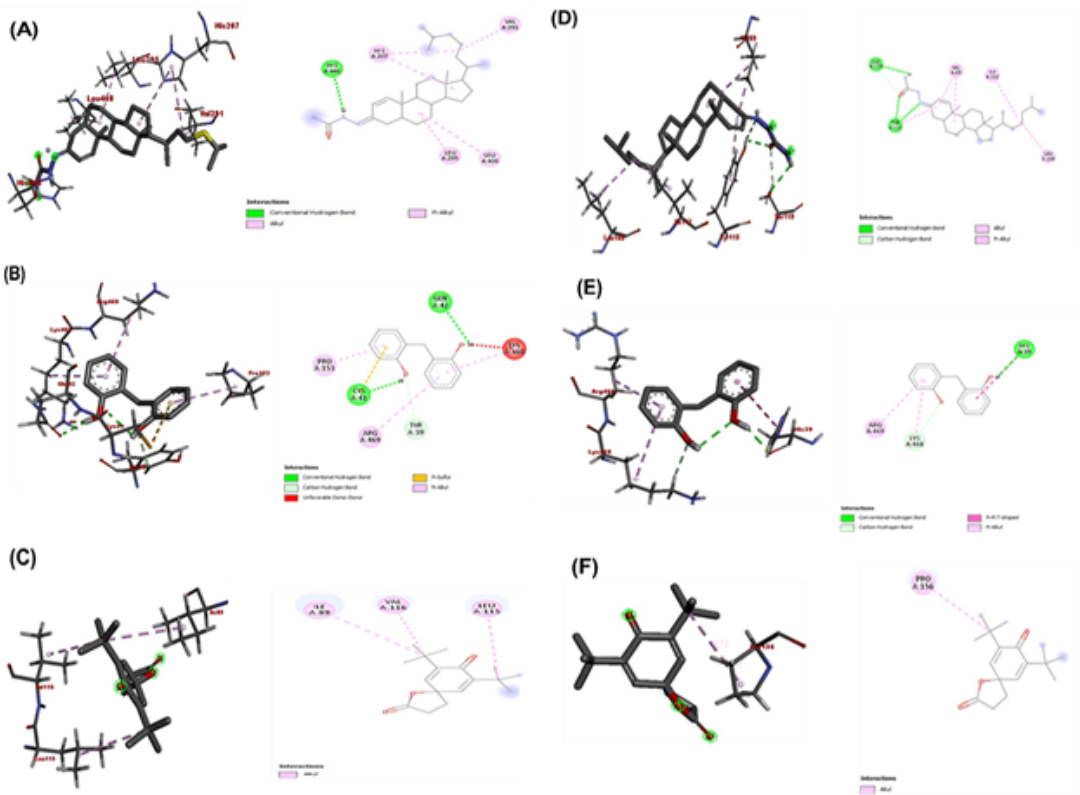


Figure 8

3D (right) 2D (left) and views of the molecular interactions of amino-acid residues of cox-1 and cox-2 with (A) 1-Coprosten-3-one semicarbazone (B) Phenol, 2,2'-methylenebis (c) 7,9-ditert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione.

Supplementary Files

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