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Integrated computational analysis, *in vitro*, *in vivo* investigation on *Myristica fragrans* Houtt. essential oils for potential anti rheumatic activities

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ABSTRACT

Rheumatoid arthritis (RA) is an inflammation associated autoimmune disorder that is prevalent worldwide. Occurrence of severe side effects with current therapeutic regimens is major concern these days. We investigated *Myristica fragrans* mace and seed for management of inflammation and RA. Oils were extracted and analyzed using ATR-FTIR conforming occurrence of the aromatic amine, phenolic, and carboxylic acid groups mostly found in terpenoids. Most of tested compounds exhibited compliance with drug likeness, bioavailability and ADMET set parameters of safety. Molecular docking analysis showed interaction (-0.6 ΔG (kJ mol^{-1}) of Myristicin with 4F5S. A significant antioxidant activity with DPPH, H_2O_2 and FRAP assays was observed in case of essential oil. Essential oil part also presented a significant protective effect during heat induced hemolysis (66 ± 1.0 %), BSA denaturation assay (61 ± 1.4 %) and higher levels of proteinase inhibition (70 ± 1.4 %). Acute anti-flammatory model in rat presented a significant decrease in inflammation with essential oil. It was concluded that *Myrictica fragrans* essential oil possess significant ($p < 0.005$) anti-inflammatory potential that can play a key role in management of RA.

1. Introduction

An important autoimmune disorder called Rheumatoid arthritis (RA) is the leading cause of immobility across the globe affecting 18 million people (GBD, 2019), with a dominant population of women (70 %) and elderly persons (55 %) (Petsch et al., 2016). RA is associated with severe pain, inflammations of joints, decreased life expectancy and substantial financial constrains (Smolen et al., 2018, Cieza et al., 2020).

Several types of autoantibodies are considered responsible for RA that mainly upset self epitope like rheumatic factors and anticitrullinated proteins (Sharma and Goel, 2023). The role of inflammation associated cytokines comprising IL-2, TNF-α, IL-13 and IL-6 are important for advancement of RA (Petsch et al., 2016). Cell inflammasomes are mainly activated through TNF-α, that accelerates release of several other inflammatory mediators and augments inflammation (Kany et al., 2018). Similarly, activated Th1 cells produce IL-2 that facilitate T cell proliferation and thus lead to localized inflammation (Shamriz et al., 2017).

Diverse RA patient management strategies are used including use of medications, exercise and change of life style (Ferro et al., 2017, Lau et al., 2018) however, occurrence of severe side effects is major concern. Further nature of heterogenic immunological mechanisms and pathological changes in RA limit the efficacy of drugs in patients (Ouboussad et al., 2019). Numerous RA phenotypes (synovial) with distinct cellular

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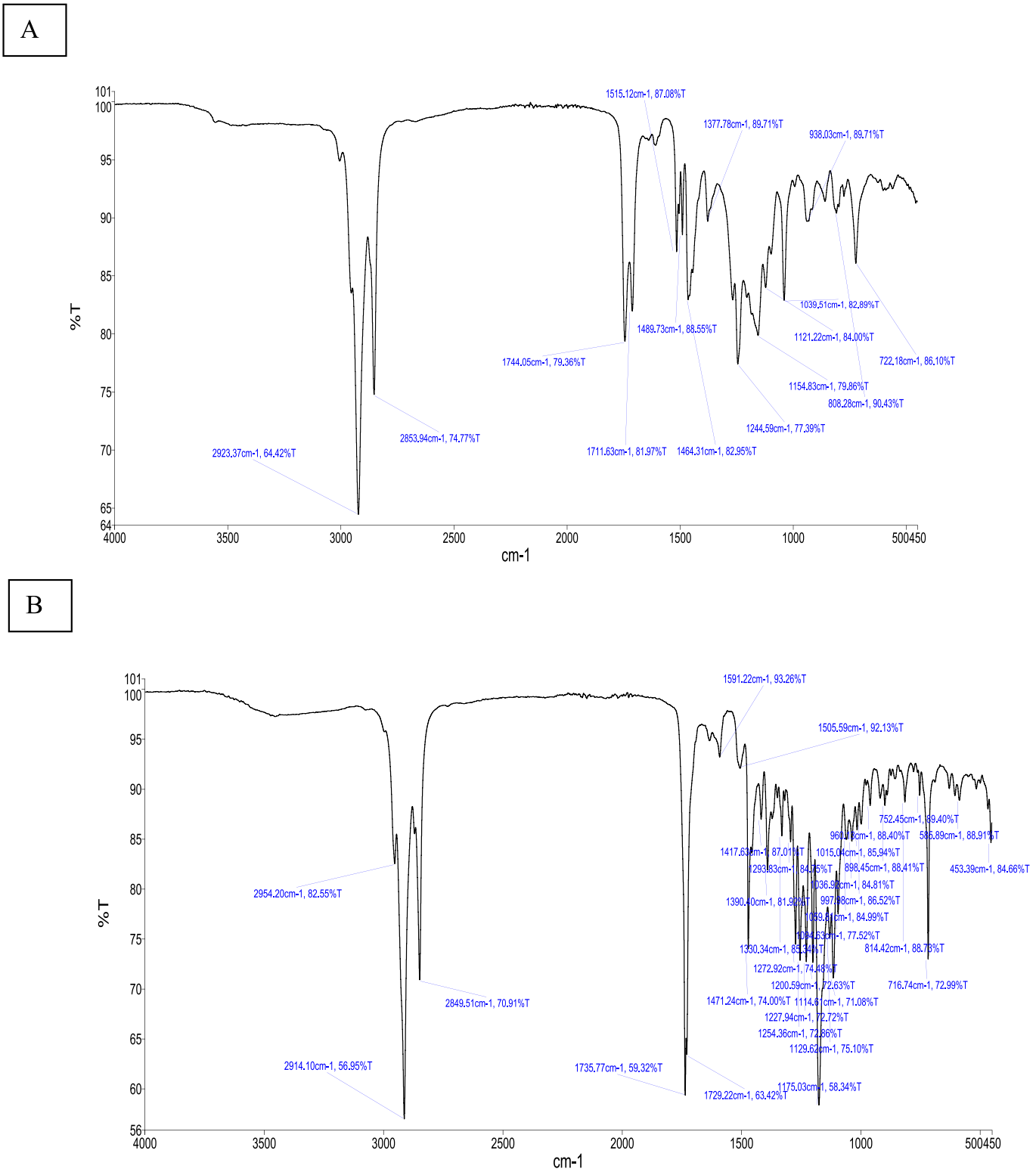

Fig. 1. FTIR analysis of nutmeg seed(A) and mace oil (B).

composition (myeloid-fibroid- lymphoid phenotypes), signaling mechanism, cell infiltration and molecular signatures are also important for consideration in management of RA (Humby et al., 2019). Medicinal plants have a centuries old traditional usage history linked with safety and efficacy (Sharma et al., 2023). More than 80 % of druggable small molecule are of natural origin and particularly extracted from medicinal plants (Almarfadi et al., 2022).

Myristica fragrans Houtt. (Myristicaceae), commonly referred as nutmeg (Jaifal and Jawatri in Pakistan). It is an evergreen tree, native to Indonesia (Maluku Islands), yet commonly cultivated in USA, South Africa, Srilanka and India (Francis et al., 2019). It is a common spice in Indian subcontinent and keeps several medicinal claims as prescribed by Ayurveda including antidepressant, antidiarrheal, antirheumatic, aphrodisiac and antiparasitic properties (Ziyatdinova et al., 2016).

Table 1
ATR-FTIR analysis of nutmeg and Mace oils.

Functional groups	Peak range (cm^{-1})	Bond	Nutmeg oil signals	Mace signals
Aliphatic Amine	3300–3500	N–H Stretching		
Alcohol/ Phenol	3200–3600	O–H stretching		
Alkanes	2850–2970 2850–2970	C–H stretching C–H stretching	2923.3 2853.9	2954.2, 2914.1 2849.5
Carbonyl	1690–1760	C–H stretching	1744.0, 1711.6	1735.7, 1729.2
Alkenes Alkyles R-C(O)–NH2 R-C(O)–NH-R	1610–1680 1590–1655 1510–1560	-C = C- Stretching -N–H bending N–H bending	1515.12	1505.5, 1591.2
Alkanes Ester (aromatic)	1340–1470 1365 + 1395 1250–1310	C–H Bending –CH $(CH3)_2$ Bending O = C-O-C stretching	1489.73, 1464.3 1377.83 1244.59	1471.2, 1417.3, 1390.4, 1330.2, 1293.8, 1272.9, 1254.3, 1227.9, 1200.5
Alcohol/ Ether/Ester /Carboxylic acid	1180–1360	C-O Stretching		
Aliphatic amines	1050–1300	C-O Stretching	1154.83,1121.2	1175.3, 1129.6, 1111.6,
Alcohol/Ester /Carboxylic acid	1050–1300	C-O		1094.2, 1059.6,
Aromatic amine	1020–1250	C-N Stretching	1039.5	1036.5,1015.7
Carboxylic	910–950	O–H bending	938.0, 808.2,722.1	960.8, 898.4, 814.2
Aliphatic bromo	500–600			716.7, 453.9

Literature review indicates presence of myristicin, D-limonene, β-myrcene, sabinene, α-pinene, β-pinene as key components of essential oil in *M. fragrans* (Ashokkumar et al., 2022). Based on traditional Ayurvedic claims, we investigated *M. fragrans* essential oil for its potential use in pain relieving formulations with the help of integrated computational approach, *in vitro* and *in vivo* validation.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents used in the project were analytical grade. Bovine serum albumin, Tris HCL, Hydrogen peroxide, $FeCl_3{\cdot}6H_2O$, Tris HCL, Trypsin (Bovine pancreas) and casein were purchased from Oxoid (Hampshire, UK) and Merck, (Dorset, UK). Folin-Ciocalteu′s reagent, carrageenan, sodium phosphate, Diclophenac sodium, DPPH and TPTZ were procured from Sigma (Sigma Aldrich, St. Louis, MI, USA).

2.2. Sampling and processing of peels

Myristica fragrans was purchased from local herbal market and samples were authenticated by taxonomist in Institute of Bio Sciences, Gomal University D.I.Khan, Pakistan. The nutmeg having two parts called mace and seeds, that were extracted separately. A defined amount of mace (50 g) was grinded to powder and placed in clevenger apparatus to extract essential oil. The hydrodistillation was carried out till 8 hrs and then essential oil was collected. The essential oil was dried by using sodium sulphate. Likewise, seeds (50 g) were grinded and extracted through solvent extraction (*n*-hexane) using soxhelet extraction apparatus. The solvent was evaporated using rotary evaporator and obtained oils were stored at −20 °C.

2.3. FTIR analysis

The FTIR analysis of both mace and seed parts was performed for better understanding of functional groups. Analysis was performed by using FTIR spectrophotometer (wave number 500–4000 cm $^{-1}$) with regular 128 scans with 4 cm^{-1} intervals. The spectra was recorded and functional groups interpretation was accomplished by using Thermofisher data base (Online).

2.4. PASS, ADMET analysis and drug likeness studies

For prediction of biological activities PASS online tool (Way2drug) was used whereas, ADMET and drug likeness properties were evaluated by several computational tools (molinspiration tool, SWISS ADME and pkCSM) (Hari., 2019, Han et al., 2019).

2.5. Ligand protein interaction (molecular docking)

Molecular docking was performed to investigate possible interaction of drug candidates with receptor protein. Bovine serum albumin (BSA) transcriptional regulators 4F5S (Cao et al., 2019) was retrieved from protein data bank (PDB) whereas three dimensional structure of molecules was downloaded from pubchem. Molecules were prepared for docking as reported previously (Rafey et al., 2022) and active site prediction was executed by CAStp tool, whereras docking was performed by using Auto Dock vina (v6.0) embedded with Lamarckian algorithm (Trott and Olson, 2010). Finally docked molecules were validated and interaction was analsyed by using Discovery studio and Ligplot^{+} tools.

2.6. Antioxidant activities

2.6.1. Hydrogen peroxide (H_2O_2) free radical scavenging activity

A modified method was used for determination of H_2O_2 based antioxidant activity (Rajamanikandan et al., 2011). Concisely, H_2O_2 stock solution (2 mM) was prepared followed by mixing (600 μL) with test sample (400 μL). The reaction mixture was vortexed and absorbance was measured after 10 min at 230 nm. As reference standard, ascorbic acid was used and results were expressed as;

$$\%Inhibition = (1 - A_0/A1) \times 100 \quad (1)$$

Where A_0 is absorbance of sample and A1 was absorbance of control.

2.6.2. DPPH assay

The antioxidant activity of samples was determined by using modified procedure (Aryal et al., 2019). Briefly, fresh DPPH (in methanol;0.5 mL; 0.1 mM) solution was combined with different concentrations of test sample (0.5 mL) and placed in dark cabinet (30 min). Later absorbance of test samples was recorded at 517 nm. The ascorbic acid was used as reference standard and results were expressed as below;

$$\%inhibitionofpeels = \%Inhibition = (1 - A_0/A1) \times 100 \quad (2)$$

Where A_0 is absorbance of sample and A1 was absorbance of control. A linear plot was used to calculate IC_{50} values of test samples.

2.6.3. FRAP assay

Ferric ion reducing power of tested sample was determined using modified method (Chandel et al., 2020). Briefly fresh FRAP reagent was set by adding TPTZ (10 mM prepared in 40 mM HCL) to acetate buffer (300 mM), and ferric chloride (20 mM) in specified ratio (10:1:1). Tested sample (100 μL) was reacted with FRAP reagent (3 mL) and placed in dark place (30 min). Afterwards absorbance was recorded (at 593 nm) by using UV–Vis spectrophotometer. The Ferrous reducing capacity was

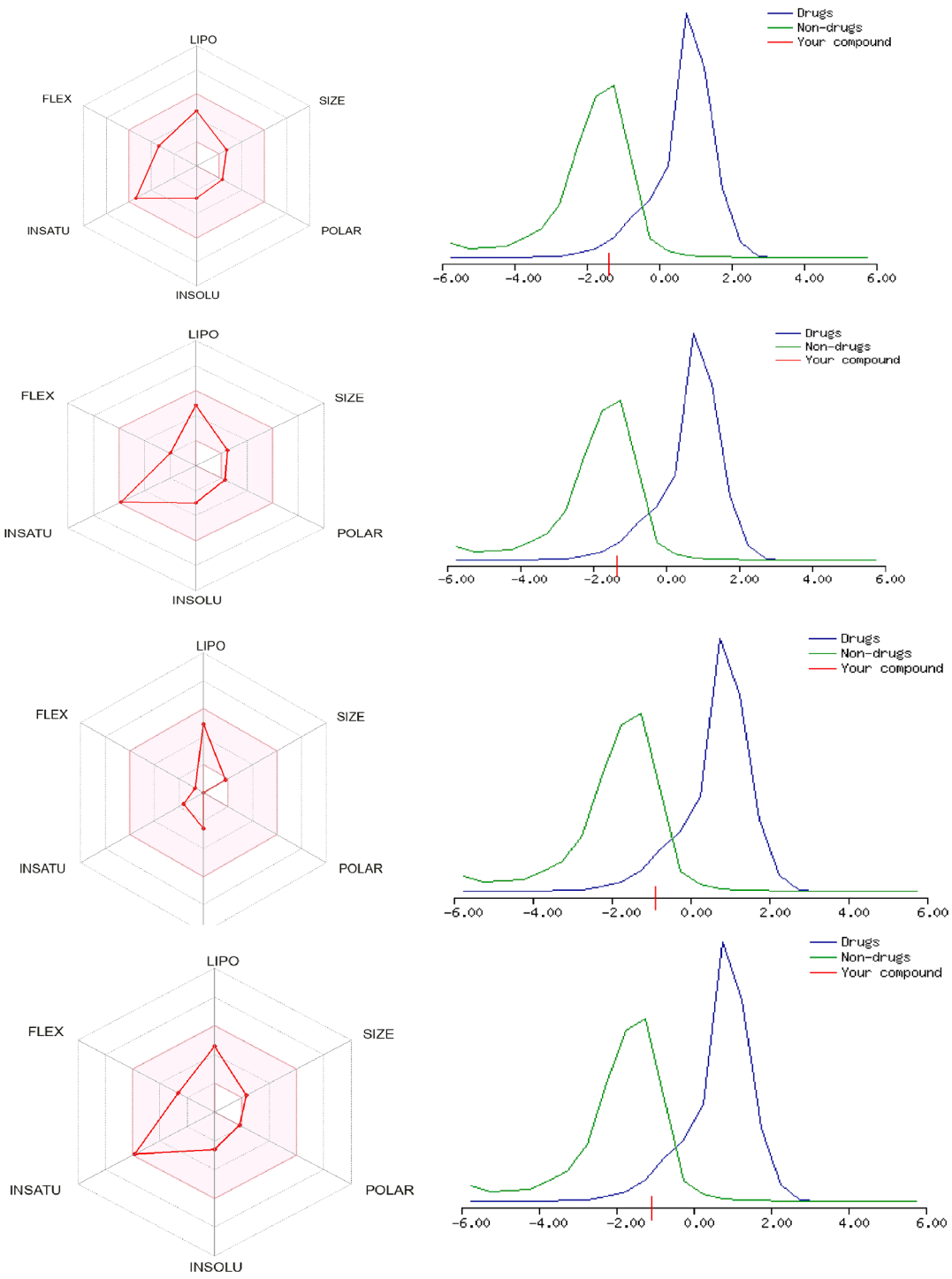

Fig. 2. Bioavailability radar and drug likeness score (-1.37) for elemicin (-1.34) for myristicin (-1 to + 1.mol), (-0.87) of Sabinene, (-0.87) of methyl eugenol (-1 to + 1.mol).

determined by $FeSO_4$ standard curve and results were expressed as μg equivalents.

2.7. Biological activities

2.7.1. BSA denaturation assay

Protein denaturation assay was executed by modified method (Gunathilake et al., 2018). Concisely, BSA (1 %, 200 μL) and test sample (20 μL) were added to phosphate buffer saline (4.78 mL, pH 6.4) followed by incubation for 15 min (37 °C). Afterwards, this solution was placed in water bath (for heating) for 5 min set at 70 °C. Later, the solution temperature was allowed to settle down at room temperature and results were recorded at 660 nm using a UV–VIS spectrometer. The diclofenac sodium was used as reference standard. Following formula was used for calculation of % inhibition, following

$$\%inhibition = (1 - A_0/A1) \times 100 \quad (3)$$

Where A_0 is absorbance of sample and A1 was absorbance of control.

Table 2
Lipinski properties of compounds.

Compound name	[Molecular Weight] <500 Da	[Log p] < 5	[H-Bond] Doner [5]	[H-Bond] Acceptor < 10	No of Violations
Elemicine	208.11	2.71	0	3	0
Hexadecanoic acid	257.11	2.68	1	3	1
Methyl Eugenol	178.10	2.59	0	1	0
Myriscitin	192.08	2.82	0	3	0
octacosane	257.11	2.68	1	3	1
Sabinene	495.32	0.67	7	8	1

2.7.2. Proteinase inhibitory activity

Proteinase inhibitory potential was determined by using standard procedure (Sakat et al., 2010) with slight modifications. Briefly, 0.04 mL of test sample was reacted with 0.012 mg trypsin, pre dissolved in 2 mL Tris-HCl buffer (pH7.4, 0.02 M) and placed in oven at 37 °C for 5 min. Afterwards casein (0.8 %, w/v, in 2 mL buffer) was mixed with reaction mixture and incubated further (20 min). the reaction was stopped by adding perchloric acid (70 %) followed by centrifugation. Results was recorded by measuring absorbance of supernatant solution (210 nm). Following equation was used for calculating anti-proteinase activity for all the samples.

$$\%inhibition = (1 - A_0/A_1) \times 100 \quad (4)$$

Where A_0 is absorbance of sample and A_1 was absorbance of control.

2.7.3. Heat-induced hemolysis

Heat induced heamolysis was performed by previously adopted method (Sakat et al., 2010). Blood suspension 0.05 mL was added to 0.05 mL of test sample followed by addition of 2.90 mL of phosphate buffer saline (PBS).The mixture was mixed slightly and incubated in water bath for 20 min at 54 °C. Afterwards, the reaction mixture temprature was settle to lower till room temprature and centrifuged at 2500 rpm (3 min). The supernatant was collected (1 mL) and added to 1 mL PBS followed by measurement of absorbance at 540 nm. Percent inhibition was calculated by following formula;

$$\%inhibition = (1 - A_0/A_1) \times 100 \quad (5)$$

Where A_0 is absorbance of sample and A_1 was absorbance of control.

2.8. In vivo acute anti-inflammatory activity

Anti-inflammatory activity was performed in pathogen free experimental animals (rats) (Maswaseh et al., 2006, Zhang et al., 2022). The ethical approval for the animal trials was obtained from Ethical Review Board (# 246/QEC/GU, Year 2020). The animals were obtained from NIH Islamabad (Pakistan) and acclimatized for 1 week in standard lab environment. The right-hind paw of each animal received an injection of 1 % carrageenan solution (0.1 mL) in normal saline to induce inflammation. Before injecting carrageenan, all preparations were applied in a total amount of 0.3 g, rubbed 50 times with the index finger. The oil as well as was diclofenac emulgel (2 g) was administered similarly. After injecting carrageenan intraplantarily, as well as 1, 2, 3, and 4 h later, the edema value was assessed using a vernier caliper. Equation (below) was used to calculate the mean percentage of inflammation reduction:

$$\%reduction = (CG - Treated/CG) \times 100$$

2.8.1. Animal grouping

The rats (n = 6) used during investigation were grouped into 4 groups.

Group-1 Given Normal saline 10 mL/kg.

Group-2 Given 0.1 mL of 1 % Carrageenan solution in normal saline

Table 3
ADMET Properties for compounds.

Properties	Compounds					
	Elemicine	Hexadeca-noic acid	Methyl Eugenol	Myriscitin	octacosane	Sabinene
TPSA [A^0]	27.69	37.30	18.46	27.69	0.00	144.85
Consensus Log $P_{O/W}$	2.58	5.20	2.58	2.49	10.81	0.56
Absorption						
Water solubility [logmol/L]	−3.096	−5.324	−2.793	−3.245	−7.085	−2.926
$CaCO_2$ permeability [log Papp in 10^{-6} cm/s]	1.6	1.557	1.556	1.469	1.114	−0.089
Intestinal absorption [human] [% absorbed]	96.861	91.957	95.948	97.684	86.922	50.205
Skin Permeability [log Kp]	−2.23	−2.371	−1.875	−2.518	−2.743	−2.736
P-glycoprotein substrate	No	No	No	No	No	Yes
P-glycoprotein I inhibitor	No	No	No	No	No	No
P-glycoprotein II inhibitor	No	No	No	No	Yes	No
Distribution						
VDss (human) [log L/Kg]	0.199	−0.56	0.287	0.094	0.181	−0.611
Fraction unbound (human) [Fu]	0.291	0.094	0.281	0.268	0.043	0.483
BBB permeability [logBB]	0.44	−0.119	0.486	0.293	1.166	−1.757
CNS permeability [log PS]	−2.101	−1.816	−1.922	−2.149	−0.762	−6.15
Metabolism						
CYP2D6 substrate	No	Yes	No	No	No	No
CYP3A4 substrate	No	Yes	No	No	Yes	No
CYP1A2 inhibitior	Yes	Yes	Yes	Yes	Yes	No
CYP2C19 inhibitior	No	No	No	No	No	No
CYP2C9 inhibitior	No	No	No	No	No	No
CYP2D6 inhibitior	No	No	No	No	No	No
CYP3A4 inhibitior	Yes	No	No	No	No	No
Excretion						
Total Clearance[logml/min/kg]	0.366	1.763	0.344	0.199	2.119	0.093
Renal OCT2 substrate	No	No	No	No	No	No
Toxicity						
AMES toxicity	Yes	No	No	No	No	No
hERG I inhibitor	No	No	No	No	No	No
hERG II inhibitor	No	No	No	No	Yes	No
Hepatotoxicity	No	No	No	No	No	No
Skin Sensitisation	Yes	Yes	Yes	No	Yes	No

Table 4
Docking analysis against targets.

Compound name	Binding free energy (ΔG) [kJ mol^{-1}]	Rank of Pose	No of H bonds	Residues for H-Bond Interaction	Residues of other interactions
4F5S					
Elemecin	−5.7	2	1	Arg435	Thr190, tyr451, Ala193, Leu454, Leu189, Ser428, Ile455
Myristicin	−6.6	1	1	Arg256	Arg217, Tyr149, Ser286, Ile289, Ala260,Ile263, Leu237, Lys221, Leu218
Sabinene	−4.6	1	–	–	Lys131, Val43, Phe36,Lys20, Leu24, Val40, Gly21, Trp134

or 1 % Carboxy methyl cellulose 0.1 mL.

Group-3 Given 2 g of commercial diclofenac gel (1 % Voltral® Emulgel) was used as an anti-inflammatory reference drug.

Group-4 Given Test oil.

2.9. Statistical analysis

All experiments were performed in triplicates and data was presented as ± SD. The Pearson correlation and regression analysis was used during investigation with $p < 0.05$ to express the data.

3. Results

3.1. Percent yield calculation

The Mace essential oil yield was calculated as 1.2 % whereas seed oil percent yield was 2.6 %.

3.2. FTIR analysis

The presence of the aromatic amines, phenolic groups, and carboxylic acid functional groups were seen in Mace part. Notably a multifaceted FTIR spectrum was documented showing existence of assorted functional groups (Fig. 1, Table 1). Alkane signals were observed at 2849.5 to 2954.2 cm^{-1} owing to C–H stretching. Correspondingly signals within 1735.2—–1729.2 cm^{-1} range were attributed to carbonyl groups conferred because of C–H stretching.

In case of seed oil, spectrum was equally complex and a bit tilted since alkane signals were seen from 2853.9 to 2923.3 cm^{-1}, occurred due to C–H stretching. Also signals appeared due to carbonyl groups were seen at 1711.6–1744.0 cm^{-1} (C–H stretching) (Fig. 2, Table 1).

3.3. Drug likeness (Lipinski's rule)

The drug like properties of *M. fragrance* major compounds were determined by using Lipinski's rule of five (Molinspiration tool) (Table 3). Outcome of myristicin, elemecin and methyl eugenol were in complete agreement with Lipinski's rule and their drug likeness scores were within permissible limits with only slight variations (-1 to + 1). Further bioavailability criteria of these compounds (set in SWISS ADME) was in agreement with normal limits (Fig. 2, Table 2).

3.4. ADMET properties and bioavailability

The ADMET results of all analsyed components were in settlement with standard ADMET analysis. All tested compounds were reported as water soluble (log mol/L −2.96 to −7.085) and having poor lipid solubility (Table 3). All analyzed compounds presented significant absorbtion (>80 %) from gastrointestinal tract except sabinene (50.20 %). Most of compounds were neither substrate nor-inhibitors of P-glycoprotein. The drug distribution in plasma is mainly assessed by VSss (distribution volume at steady state), and moderate distribution of compounds was recorded (Table 4). Elemecin and methyl eugenol were reported to cross blood brain barrier (logBB > 0.3) and therefore they may have effect on CNS. Detailed ADMET analysis is shown in Table 3.

3.5. Ligand protein interaction (molecular docking)

Transcription regulator 4F5S (for BSA) was used for elemecin, myristicin and sabinine interaction in docking (Table 4). Significantly higher binding energy (-0.6 ΔG (kJ mol^{-1}) was noticed in case of Myristicin a stronger H-bond formation was noticed with Arg256 residue. Here, non-hydrogen bonding interactions were observed with amino acids including Lys221, Arg217, Ile289, Leu218, Tyr149, Ser286, Ile263, Ala260 and Leu237. Likewise, in case of elemecin Arg235 was able to form a strong H-bond with comparably less free energy (-5.7 ΔG (kJ mol^{-1}) and other interactions including π-sigma and π- π were seen with Leu189, Thr190, Ser428, Tyr451, Ala193, Ile455 and Leu454 (Fig. 4). No interaction of sabinene was noticed that shows little or no participation in biological activity (Fig. 3). It was thus established that myristicin and elemecin can interact with BSA.

3.6. Antioxidant assays

The antioxidant activities were assessed using DPPH, H_2O_2 and FRAP assays. MEO presented significant DPPH inhibition (IC_{50} 42.3 μg/mL) whereas Mace was inactive. Similarly, significant inhibition of H_2O_2 was recorded in case of MFEO (54 ± 0.21 %) compared to mace oil (44 ± 0.45 %). Similar trend was noticed in FRAP estimation where a high FRAP value (14.11 μg) was recorded compared to Mace (14.11 μg) (Table 5).

3.7. Biological assays

Various biological assays were performed on tested samples to investigate *in vitro* anti-arthritic actvities. Highest protection of blood cells was recorded in case of MFEO (66 ± 1.0 %) compared to mace oil (38 ± 1.2 %). Similarly significant results were observed in protein denaturation assay, where MFEO presented better results (61 ± 1.4 %) compared to mace oil (33 ± 2.3 %). Similar trend was also recorded in case of protenase inhibition assay where MFEO showed highest inhibition (70 ± 1.4 %) compared to mace oil (60 ± 1.3 %)(Table 6).

3.8. Correlation studies

Person correlation and regression analysis was used for antioxidant anti-inflammatory activities. A positive ($r^2 = 0.955$) correlation was seen (p value 0.04) with DPPH- protein denaturation assay (Supplementary data). A similar, positive correlation was recorded ($r^2 = 0.96$, p value 0.03) in correlation studies of DPPH-proteinase inhibition assay. Analogous trends were documented with DPPH-Heat induced hemolysis (Supplementary data).

3.9. In vivo acute inflammation

Statistical analysis with ANOVA one way and *post hoc* Dunnett's test application has shown a momentous repression in paw edema with oil as well as diclofenac gel after 1 h (**$p < 0.001$), 2, 3 and 4 h (***$p < 0.001$) as compared to carrageenan group. Additionally a considerably significant % inhibition in rat paw edema was also observed over a period of 4 h with both oil and diclofenac gel application (Fig. 4).

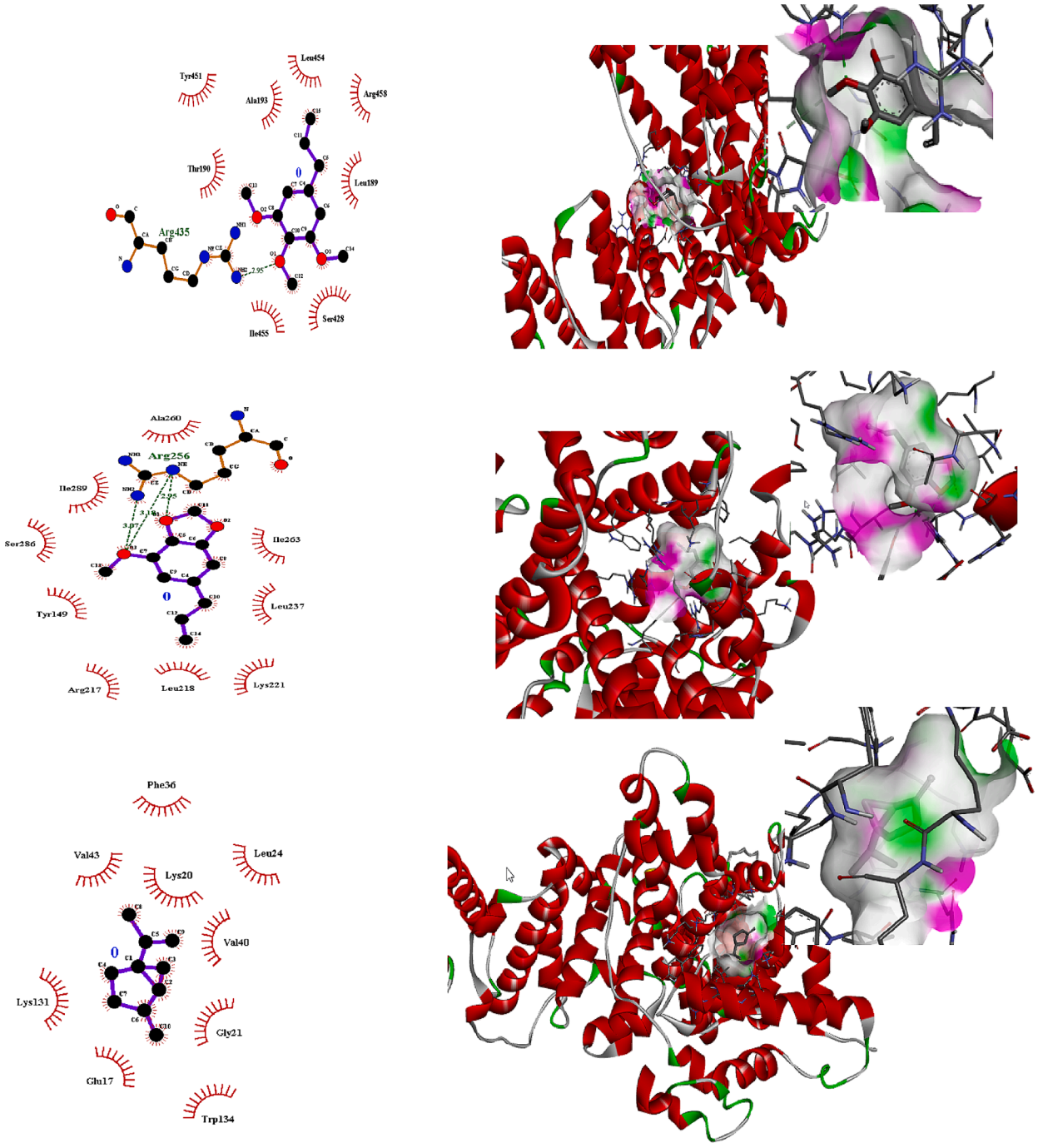

Fig. 3. Elemicin's (pose 2), myristicin pose rank 1, and sabinine pose 1 interacts with the binding sites inside of the transcriptional regulator 4F5S in a 3D and H, non-H bonding mode.

4. Discussion

Myristica fragrans is a common spice and obtained as fruit. The fruit is further comprised of kernel, mace (scarlet aril) and nut. Both mace and nut parts are commonly used for multiple ailments in ancient Ayurveda (Sultan et al., 2023). We investigated both essential oil and fixed oil from mace and nut parts for anti-arthritic activities. Initially ATR-FTIR analysis was performed that showed a complex spectrum appearing due to mingling of diverse vibrational modes and signal overlapping (Agatonovic-Kustrin et al.,2020). In mace oil N–H and O–H bending were detected in signals arising from 1505.5 – 1591.2 cm^{-1} were likely due to R-C(O)–NH-R -N–H bending, while ester (aromatic) groups were spotted due to O = C-O-C stretching and –$CH(CH3)_2$ bending signals ranging from 1200.5 to 1390.2 cm^{-1}. Typical peaks for monoterpenes and C = O stretching, were also recorded that are typical for essential oils containing terpenoids (Oliveira et al., 2016). Seed oil FTIR spectra revealed R-C(O)–NH-R groups at 1515.12 cm^{-1} that occurred due to N–H bending. Similarly alkanes groups were detected due to C–H bending with signals at 1489.73 and 1464.3 cm^{-1}. In addition, aromatic moieties that occurred because of –$CH(CH3)_2$- bending were noticed at 1377.3 cm^{-1}. Presence of carboxylic acids (O–H bending) at 808.2, 722.1, 938.0 cm^{-1}, aromatic amines (C-N Stretching) at 1039.5 cm^{-1} and aliphatic amines (C-O Stretching) at 1154.83 were also documented as reported earlier in case of essential oils (Agatonovic-Kustrin et al., 2020).

The component analysis was performed by earlier investigations

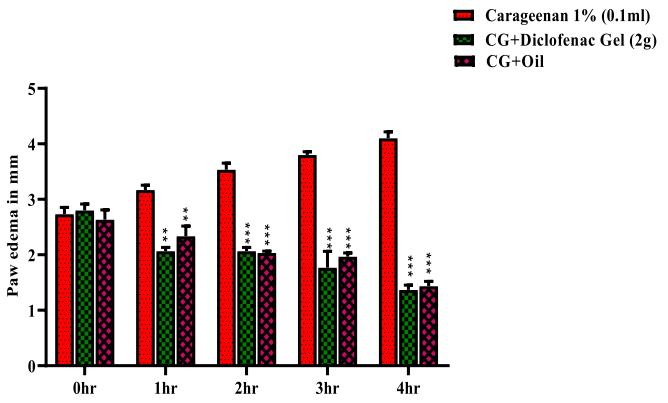

Fig. 4. *In vivo* anti-inflammatory activities of tested samples of *Myristica fragrans*.

Table 5
Antioxidant activity of essential oil and Mace obtained from *Myristica fragrans*.

Serial No.	Sample name	DPPH IC_{50}µg/mL	H_2O_2 % inhibition*	FRAP value µg
1.	Mace (fat)	In active	44 ± 0.45 %	10.71
2.	Oil	42.3	54 ± 0.21 %	14.11
3.	Ascorbic acid	15.3	61 ± 1.2 %	253.5

*0.2 mg/mL.

Table 6
Anti arthritic potential of essential oil and Mace obtained from *Myristica fragrans* (±SD).

Serial No.	Sample name	Heat Induced hemolysis % inhibition	Protein denaturation	Proteinase inhibition
1	Mace (fat)[1]	38 ± 1.2	33 ± 2.3 %	60 ± 1.3 %
2	Oil[2]	66 ± 1.0	61 ± 1.4 %	70 ± 1.4 %
3	Ascorbic acid[3]	72 ± 1.2	57 ± 1.2 %	94 ± 1.6 %

1.5 mg/mL[1], 0.05 mg/mL [2], 0.01 mg/mL [3].

(Matulyte et al., 2019) and major components including elemecin, methyl eugenol, myristicin, sabinene, hexadecanoic acid and octacosane were selected for *in silico* analysis. In first instance all components were analysed for drug likeness. Lipinski's parameter for all tested molecules were in compliance with drug likeness except slight violations in case of hexadecanoic acid, octacosane and elemecin. Further ADMET analysis was performed for all molecules. Topological polar surface TPSA range is considered as outstanding if it is less than 100 and this shows admirable oral absorption or permeability through membrane (Qidwai, 2016). Except sabinene, all tested molecules were within speculated limits. The $CaCO_2$ permeability, P-glycoprotein substrate or inhibitors are very important to estimate absorption of molecules. As obvious from previous reports if Papp coefficient is found more than 8×10^{-6} (absorbtion will be larger than 0.90) showing greater $CaCO_2$. We noticed lesser $CaCO_2$ levels that indicated low permeability. Likewise, <30 % oral absorbtion values indicates a poor oral absorbtion, however our molecules were provided with higher absorbtion levels (>90 %). All analyzed molecules had a slightly higher value compared to standard value for logKp, and an inconsequential disagreement in permeation through skin was recorded. Molecules with a logKp greater than −2.5 are ordinarily considered to posess lower levels of skin permeation. P glycoproteins are pivotal in removal of unwanted molecules out from cells. Excluding octacosane (inhibitor), no compound was either substrate or inhibitor that shows selected metabolism.

The CNS-permeability and (logBB), % unbound (human), distribution volume (VDss), values are very helpful in determination of drug distribution in body tissues. Based on standard readings, tested molecules had low VDss values that shows low drug distribution. The logBB value, explains effect of drug on CNS and a value > shows 0.30 that drug candidate can cross membrane (BBB). The elemicin and methyl eugenol were able to have effect on CNS. Similarly all molecules were noticed to be metabolized in liver based on findings of cytochrome P450.

Ligand-protein interaction analysis was performed by molecular docking. Major components were docked against tracriptonal regulator 4F5S and Myristicin showed a higher affinity (-0.6 ΔG (kJ mol^{-1}). This interaction was mainly supported by one hydrogen bonding and hydrophobic interactions with nine amino acid residues. The specificity in ligand binding interaction is mainly regulated by hydrogen bonding interactions (Wade and Goodford, 1993, Patil et al., 2010). In case of hydrophobic interactions π-sigma, vandervaal's interactions were of major importance. The hydrophobic interactions are rendered as very crucial for biological activities (Hansch and Klein, 1991) therefore myristicin was considered as biologically active against 4F5S. Elemecin also presented significant hydrophobic interaction with amino acid residues of 4F5S and therefore it was concluded that both myristicin and elemecin can contribute towards inhibition.

Free radicals are of prime importance in health related issues since they are highly unstable (Gupta et al., 2014) and a slight imbalance in oxidative stress leads to development of serious diseases including rheumatoid arthritis (Fotina et al., 2013). We investigated effect of tested samples of *Myristca fragrans* using antioxidant assays (DPPH, H2O2, and FRAP) with diverse mechanisms. A strong antioxidant activity was recorded with essential oil part compared to Mace part(Seed). This can be attributed to presence of monterpenes and phenolic groups (Dosoky and Setzer et al., 2018).and it have been proven already that these essential oils react through addition H atom and inhibition of free radicals chain in reaction (Amorati et al., 2013, Atere et al., 2018). Various previous investigations also supported strong antioxidant potential of *M. frangrans* oil (Matulyte et al., 2020).

Several *in vitro* assays were performed to evaluate the antirehumatic effect of both tested samples. Protein denaturation plays a key role in advancement and development of RA since this effects results in development auto antigens on a dissimilar manner (Phusricom et al., 2013). In protein denaturation, secondary and tertiary structural changes occur due to changes in strength of electrostatic forces (Modi et al., 2019). The essential oil inhibited protein denaturation significantly, that was attributed to presence of synergistic effects of mono terpenoids (Matulyte et al., 2019). Various reports have confirmed that tissue damage in body is mainly due to proteinases during the inflammatory progressions. Thus inhibition of proteinases can be helpful in management of inflammatory responses (Chandra et al., 2012). Both tested samples significantly inhibited proteinase enzyme and therefore may contribute towards lowering inflammation in RA. Presence of several biologically active components in essential and fixed oil parts may be contributing towards strong anti-inflammatory properties through significant antioxidant activity and interference with enzymatic pathways (Gunathilake et al., 2018). Inflammatory modulation is also explained by release of lysosomal contents due to breakage (Chopade et al., 2012). Because of close resemblance of RBCs with lysosomes (Govindappa et al., 2011) we determined the protective effect of tested samples on heat induced hemolysis. It was evident that essential oil part of *M. fragrans* showed significant protective effect and this was mainly due to its capability to interfere with Ca + ions influx in the RBCs (Omale and Okafor, 2008). Finally, to validate the *in silico* and *in vitro* analysis, animal models were used. One way ANOVA was used based on number of variable used and to signify the decrease in paw edema in different animal groups as mean of three independent experiments. One way ANOVA was followed by *post hoc* Dunnett's test, since one of the tested animal groups served as control. A marked decrease in the paw diameter

was noticed compared to standard drug that confirmed antiflammatory (Rajendran and Kumar, 2010) effects of essential oil, that may have potential use in RA.

5. Conclusions

Essential oils are considered very important for management of diversified health concerns including rheumatoid arthritis. Due to presence of low M.wt compounds and high penetration power, several topical applications are added with essential oils for quick and sustained relief. We investigated *M. fragrans* and it was concluded that mace essential oil posess significantly inhibited the oxidative stress and anti-flammatory markers *in vitro* through its protective effects. A significant anti-inflammatory potential was observed during *in vivo* investigation that suggested a suitable usage of *Myristica fragrans* essential oil for management of rheumatoid arthritis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical statement

Ethical approval for the involvement of all animals in this study was granted by the Animal Ethics Committee Gomal University, Pakistan (No. 246/QEC/GU).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2024.103177.

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